Volume 34, Number 2, October 1961

The Yale Journal of Biology & Medicine

75 **FLUID INTERCHANGE AT THE PULMONARY CAPILLARY WALL IN LUNG EDEMA** *Frank D. Gray, Jr. and Albert S. Field, Jr.*

97 **OPTO-SEXUAL REFLEX IN THE DUCK: PHYSIOLOGICAL AND HISTOLOGICAL ASPECTS** *Jacques Benoit*

117 **APPOINTMENT BREAKING IN A PEDIATRIC CLINIC** *Robin F. Badgley and Marilyn A. Furnal*

124 **THE EFFECT OF ETHYL ALCOHOL ON NITROGEN EXCRETION IN THE RAT** *Gerald A. Klatskin*

144 **MESONEPHRIC DUCT CYST IN A NEWBORN** *Leo H. von Euler*

149 **BOOK REVIEWS**

New Alvodine®

Brand of piminodine ethanesulfonate *ethanesulfonate*

An analgesic for

1. Ambulatory patients.

Whenever codeine or codeine-aspirin combinations are used, Alvodine taken orally can relieve pain better without dulling mental acuity or enervating the patients.

2. Postoperative patients.

Alvodine injected parenterally is as potent as morphine, but unlike morphine, it permits patients to remain alert postoperatively. It favors early mobilization of patients because it is usually not hypnotic.

3. Cancer patients.

Alvodine produces relief without weakening the cancer patient. Alvodine allows patients to eat normally, to maintain their strength and to continue their day-to-day activities to the extent permitted by their disease.

Before prescribing be sure to consult Winthrop's literature for information about dosage, possible side effects and contraindications.

Alvodine is the first narcotic analgesic with action approaching "pure" analgesia. It relieves pain without producing sedation, drowsiness or drugged sensation.* This unique characteristic makes Alvodine the most desirable agent for pain relief in these three large groups of patients.

Alvodine Tablets, 50 mg., scored.
Alvodine ampuls, 1 cc. containing 20 mg. per cc.
Narcotic blank required.
Write for detailed information on clinical experience, addiction liability, side effects and precautions.

*In more than 90% of patients

Winthrop LABORATORIES
New York 18, N.Y.

CONTENTS

75 **FLUID INTERCHANGE AT THE PULMONARY CAPILLARY WALL IN LUNG EDEMA** *Frank D. Gray, Jr. and Albert S. Field, Jr.*

97 **OPTO-SEXUAL REFLEX IN THE DUCK: PHYSIOLOGICAL AND HISTOLOGICAL ASPECTS** *Jacques Benoit*

117 **APPOINTMENT BREAKING IN A PEDIATRIC CLINIC** *Robin F. Badgley and Marilyn A. Furnal*

124 **THE EFFECT OF ETHYL ALCOHOL ON NITROGEN EXCRETION IN THE RAT** *Gerald A. Klatskin*

144 **MESONEPHRIC DUCT CYST IN A NEWBORN** *Leo H. von Euler*

149 **BOOK REVIEWS**

THE YALE JOURNAL
OF BIOLOGY AND MEDICINE

Founded in 1928.

George H. Smith; *Editor-in-Chief, 1928-49; Editor-in-Chief Emeritus, 1949-52.*

BOARD OF EDITORS

Faculty

Paul B. Beeson, *Editor;* Donald L. Buchanan; Lawrence R. Freedman; Mark A. Hayes; Marie-Louise Johnson, *Assistant Editor;* Frederick G. Kilgour, *Managing Editor;* James W. Lewis, *Advertising Manager;* Vernon W. Lippard

Students

Millard J. Amdur; Sidney MacD. Baker; David M. Bull; Stephen J. Fricker; Susan Goldhor; John F. B. Haney; Martin J. Kligerman; Richard E. Marshall; Malcolm S. Mitchell; Jonathan J. Russ; Peter V. Tishler; Sigrid L. Tishler; Alfred J. Wise

---

Subscriptions. THE YALE JOURNAL OF BIOLOGY AND MEDICINE is published in August, October, December, February, April, and June from 333 Cedar Street, New Haven 11, Connecticut, U.S.A. The subscription price for one volume of six issues is $9.00 in the United States and other countries. For students registered in any school of Yale University, the subscription price is $4.00 per volume. Single copies are $2.00 each except for special issues.

Entered as second-class matter 11 January 1929, at the post office at New Haven, Connecticut, under the Act of 3 March 1879.

PRINTED BY THE VAN DYCK PRINTING CO., NEW HAVEN, CONN.

ANNOUNCEMENT *from* APPLETON

A New Book on...

DISSEMINATION OF CANCER

PREVENTION AND THERAPY

By WARREN H. COLE, M.D., GERALD O. MCDONALD, M.D., STUART S. ROBERTS, M.D., and HARRY W. SOUTHWICK, M.D.
Department of Surgery, University of Illinois
College of Medicine, Chicago, Illinois

Every line in this new book has been written for the purpose of utilizing all available information to help the patient afflicted with cancer. Dr. Cole, past president of the American Cancer Society, and his colleagues bring years of research on the nature of cancer and its dissemination to this most important book.

October 1961 462 Pages 300 Figs. (18 in Color) $12.75

A New Second Edition of...

IMMUNITY

By SIDNEY RAFFEL, SC.D., M.D.
Stanford University Medical School

This edition reflects the increased interest in immunology on the part of surgeons, internists, and geneticists. Many new experimental methods and clinical observations have been included, which clarify and prove old concepts.... a most up to date and satisfactory text.

September 1961 625 Pages $10.00

For Sale at all Bookstores or

APPLETON-CENTURY-CROFTS, Inc. 34 W. 33rd ST., NEW YORK, N.Y.

The American Surgical Supply & Equipment Co.

316 Congress Avenue	New Haven, Conn.
1715 Barnum Avenue	Bridgeport, Conn.
300 Hancock Street	Springfield, Mass.

JOURNAL OF THE HISTORY OF MEDICINE AND ALLIED SCIENCES

The Journal aims to foster humanism in medicine and to bring about closer liaison between the arts and the sciences. It publishes scholarly papers on all phases of the history of medicine and the cognate sciences, preference being given to those set in the broad framework of general and social history. Book reviews form a regular feature, and there is also a special section of Notes and Events, dealing with the 'quick' and the long-since dead, in which particular attention is given to unusual books, research in progress, and the activities of living personalities. Once or twice a year it includes 'Historical Gleanings' directing attention to papers in journals not usually seen by medical historians but pertinent to their field.

Board of Editors: Thomas R. Forbes, *Acting Editor;* Madeline E. Stanton, Elizabeth H. Thomson, *Assistant Editors;* Dorothy M. Schullian, Cornell University, Ithaca, *Editor,* Notes and Events; Erwin H. Ackerknecht, University of Zurich; Whitfield J. Bell, Jr., Philadelphia; Edwin S. Clarke, Institute of the History of Medicine, Johns Hopkins; Genevieve Miller, Western Reserve University School of Medicine, Cleveland; F. N. L. Poynter, Wellcome Historical Medical Library, London; Fred B. Rogers, Temple University School of Medicine, Philadelphia; George Rosen, Columbia University, New York; Lloyd G. Stevenson, Faculty of Medicine, McGill University, Montreal; Warner L. Wells, University of North Carolina School of Medicine, Chapel Hill; Leonard G. Wilson.

Subscription rates: $10.00 per annum; single copies $3.00.

PUBLISHED QUARTERLY FROM THE DEPARTMENT OF THE HISTORY OF SCIENCE AND MEDICINE, YALE UNIVERSITY, NEW HAVEN, CONNECTICUT

FRANK D. GRAY, JR.*
ALBERT S. FIELD, JR.**

Department of Internal Medicine, Yale University School of Medicine

FLUID INTERCHANGE AT THE PULMONARY CAPILLARY WALL IN LUNG EDEMA†

Although pulmonary edema has been studied as an entity by clinicians and physiologists since the time of Albertini,[1] confusion about the course of events leading to the recognizable clinical signs has persisted until the present. Some of this confusion has been due to the tendency on the part of medical scientists to favor a unitary hypothesis for any phenomenon which seems to be circumscribed within a single organ and physiologic framework. By now the multiplicity of events leading to the phenomenon of lung edema has been thoroughly aired,[2,3] but there still remains a common focus for all types of pulmonary edema—the excessive accumulation of fluid in the pulmonary pericapillary tissue spaces.

In a broad sense, edema is a state in which the normal balance of fluid interchange between blood capillaries and tissue spaces or body cavities is disturbed, leading to an accumulation of plasma filtrate. Although edema is frequently a generalized phenomenon, it is most dramatic when it affects the lung and pleural spaces, the pericardial space, or the brain. This is not because of any fundamental difference in the mechanism of the edema, but rather in the nature of the target organ. Furthermore, the anatomical structure and physiological function of each organ will determine the variable importance of the numerous factors responsible for edema formation.

A few years ago, a report from this laboratory posed several questions which had to do with the role of vascular pressure, anoxia, nervous vasomotor control, and ventilation in the genesis of edema of the lung.[4] Since the studies of Welch in 1878,[5] these and other questions have guided investigators into countless experiments. The answers to these questions are gradually developing, but we are beginning to see that many of the factors which have been implicated can be quite variable, and all need not be present at any one time.

---

* Associate Professor of Medicine.
** Assistant Professor of Medicine.
† This study was supported by research grant H3263 (C1) and (C2) from the National Heart Institute, U. S. Public Health Service.
Received for publication 29 March 1961.

Copyright 1961 by THE YALE JOURNAL OF BIOLOGY AND MEDICINE, INC.

Long ago clinical observation led many observers to divide edema of the lung into acute pulmonary edema and chronic passive congestion.[6] The former is due to a complex of factors which differ according to the cause, and the latter is entirely the result of left heart falure.[4] Early studies of Welch[5] led many people to assume that all edema of the lung was a manifestation of left heart failure. This is an improper deduction from his experiments and it is doubtful that he implied an exclusive cause. Certainly the experiments of Cataldi,[7] in which injury to the right ventricle of the heart produced pulmonary edema more often than injury to the left, demonstrated that a gross disproportion in the outputs of the two ventricles was not the primary cause of pulmonary edema. Many other observations have confirmed the possibility of pulmonary edema in the absence of left ventricular failure. Cameron[3] has emphasized the inadequacy of Welch's experiments for explaining all types of pulmonary edema. Most of the pertinent data in this regard have been summarized by Luisada.[8]

Although it is evident that many different causes and many different combinations of factors are involved in edema of the lung, an analysis of the local phenomena common to all known categories of pulmonary edema should be worthwhile. This paper is an attempt to present such an analysis, drawing on published data as well as observations from our own laboratory. A modification of the Starling hypothesis accounting for the capillary fluid imbalance found in pulmonary edema will be presented and tested against available observations.

For the purpose of this discussion, lung edema may be considered a specific instance of the general phenomenon of edema. The normal balance between the hydrostatic and osmotic pressures on either side of the pulmonary capillary wall is determined by established and demonstrable hemodynamic and physico-chemical principles. There is a slight and continued loss of fluid from the capillaries, and this is removed largely by lymphatic channels, although some may be removed via airways in the case of the lung. Changes in hydrostatic or osmotic pressures, capillary surface area, diffusion or permeability, lymphatic drainage, and pulmonary ventilation may alter this balance. Even in the presence of an otherwise stable equilibrium, an overall expansion of water and crystalloids in the circulating blood may lead to excessive accumulation of fluid in the lung tissue spaces and alveoli.

METHODS

In reporting previously published work from our own or other laboratories, methodological details will not be described because they are, in most cases, well presented in the original papers. In the case of hitherto unpublished work from our

own laboratory, the following general patterns of procedure apply. Studies involving cardiac catheterization of patients were carried out in the fasting steady state using customary techniques. Cardiac outputs were estimated by the Fick principle or by an isotope dilution technique previously described.[9] Lung function studies were accomplished using standard methods and the terminology and the symbols outlined in Comroe.[10] Essentially the same techniques were used in the animal studies. The method of cannulation of bronchi and pulmonary vessels has been described.[4] All respiratory gases were analysed by the Scholander method, blood gases by the Roughton-Scholander method, electrolytes by standard laboratory technique, pH using a Cambridge research pH meter with a 37° water-jacketed glass electrode, and osmotic pressure using a Fiske osmometer. In the case of dog experiments, the anesthetic agent was Dial-urethane. Experiments on dogs in which the preliminary arterial oxygen saturation was below 90% or pH below 7.30 were discarded on the grounds that such dogs had unpredictable abnormalities. All dogs were breathing spontaneously, either room air or oxygen.

OBSERVATIONS AND DISCUSSION

The physical factors involved in the accumulation of fluid in the extra-capillary tissue may be simply stated under four general headings: (i) the transmural (net) hydrostatic pressure; (ii) the net osmotic pressure; (iii) rates of filtration and lymph flow; and (iv) the total amount of circulating crystalloids and water. Transmural hydrostatic pressure is a function of arterial and venous pressure, arteriolar tone, venous tone, blood velocity and tissue hydrostatic pressure. The character of the tissue itself, i.e., rigid or compliant, will have a pronounced influence on hydrostatic pressure. The net osmotic pressure is related to the osmotic pressure of the intra- and extra-capillary fluids and the capillary membrane permeability. Filtration is determined by the physical properties of the membrane and the net filtration pressure. The electrical potential effect of the capillary membrane may be subsumed under filtration. Temperature gradients across the capillary membrane are probably significant, but little is known of their influence on edema. Lymph flow is a complex function, the obvious factors being the distribution of lymphatic channels (Fig. 1), tissue pressure, muscular activity, and venous pressure at the point of discharge of the lymphatic into the venous system. The rate of lymph removal, by its influence on tissue pressure, will affect hydrostatic pressure. The total amount of crystalloid solution is also a complex function, the chief factor being renal regulation of salt and water excretion. The areas in which excessive accumulation of extra-capillary fluid may occur in the lung are illustrated in Figure 1. They are the bronchial lumen, the bronchial wall, the peribronchial tissue spaces, the alveolar wall, the

alveolar space, the interstitial space of the lung, the visceral and parietal pleura, and the pleural space.

Normally, homeostatic mechanisms keep osmotic pressure and the volume of circulating water and crystalloids reasonably constant; a reduction in lymph flow, although probably important in the gradual accumulation of edema in passive congestion, seems inadequate to account for the rapid development of massive acute pulmonary edema. One first looks, therefore,

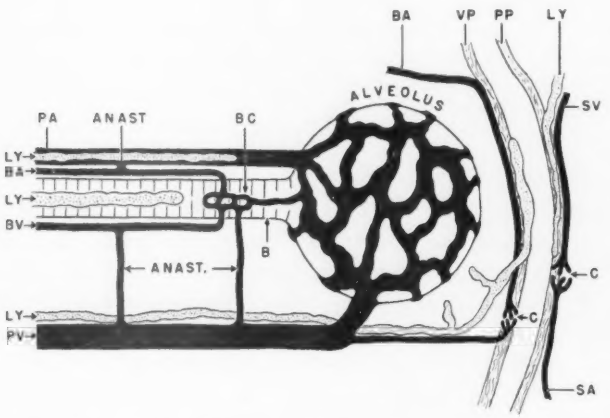


FIG. 1. Schematic representation of fluid exchange areas. Anast. = anastomoses; C. = capillary; B. = bronchus; B.A. = bronchial artery; B.V. = bronchial vein; LY = lymphatic; P.A. = pulmonary artery; P.C. = pulmonary capillary; P.P. = parietal pleura; P.V. = pulmonary vein; S.A. = systemic artery; S.V. = systemic vein; V.P. = visceral pleura.

to hydrostatic pressure phenomena as major factors in edema formation, knowing that changes in the rate of blood flow and vasomotor tone can take place, for example, with varying amounts of physical activity.

Assuming laminar flow in a uniform tube, a simplified expression of the factors involved in our use of the term hydrostatic pressure has been made by Green:[11]

$$E = Z + P + \frac{V^2}{2g},$$

where E is total energy; Z is potential head energy (height of the point above a reference plane); P is pressure head energy; V and g are velocity and gravity respectively; and $V^2/2g$ is the velocity energy or velocity head.

In this expression, Z may be ignored, since it approaches zero as the position and the reference planes are nearly identical when considering transmural pressures. Thus we can write:

$$E = P + \frac{V^2}{2g}.$$

From this relationship it is clear that pressure head energy (exerted in all directions, hence laterally against the vessel wall) is directly related to total energy and inversely to velocity. Frictional loss, however, affects energy as follows.

$$F = (P_1\text{-}P_2) + \frac{V_1^2\text{-}V_2^2}{2g},$$

where F is frictional loss; and the expression $(P_1\text{-}P_2)$ refers to energy loss from up-stream to down-stream.* As F approaches zero, pressure and velocity losses become minimal.

Thus for an ideal system with laminar flow we can say that if the total energy at the beginning of the pulmonary capillary bed remains constant, the lateral pressure will rise provided frictional energy loss or velocity decrease. The latter would occur if resistance beyond the capillary were to increase with constant precapillary energy; if the resistance increases, flow decreases. As the volume flow decreases, so will the velocity in the vascular channels proximal to the increased resistance, and the lateral pressure will rise. The frictional energy loss within the capillary diminishes if the viscosity of the blood falls, or if the ratio of endothelial surface area to moving blood volume decreases as when the diameter of a tube increases. For our purposes friction, including viscosity and vessel diameter, is considered part of resistance, but capillary resistance and post-capillary resistance will have opposite effects on capillary lateral pressure.

As lateral pressure rises, it will tend to distend the vessel, increasing the diameter, and frictional loss will diminish. A further increment to lateral pressure will thus develop, although much of the energy "saved" by less resistance will be used as velocity. A drop in resistance with an increasing pressure has been demonstrated.[12-14] This probably reflects the great distensibility of the capillary with only slight pressure changes until maximal distention occurs, after which the vascular pressure-flow relationship becomes linear.[15]

Since transmural pressure is the difference between capillary pressure and tissue pressure, the latter deserves consideration. It is probably not correct to consider the tissue pressure equal to intra-alveolar pressure, since

* As in the energy equation above, and for the same reason, Z is ignored once more.

there is a potential space between the capillary and alveolar walls. The alveolus is a polyhedron, with surface forces approximating those of a sphere and its wall has surface tension; thus, its radius is constantly tending to get smaller. According to the law of Laplace, the tension is proportional to the product of the pressure exerted on the wall times the radius. The pressure is the net pressure, the difference between the external and internal pressures. Since at the end of inspiration or expiration, the pressure inside the sphere is atmospheric (P_1), we can formulate an expression for the outside or tissue pressure (P_2):

$$T = (P_1\text{-}P_2) \times R,$$

where T is surface tension; P_1-P_2 the difference between pressure inside and outside the alveolus; and R the radius.

In this case P_1 is atmospheric and P_2 below atmospheric. As R gets smaller, either T gets smaller or P_2 (tissue pressure) gets more negative with respect to atmospheric pressure. In the latter case, the effective transmural filtration would increase. The converse would hold as R increases, and this might account to some degree for the relative infrequency of clinical pulmonary edema in emphysema. As the radius of an alveolus gets smaller, however, there must be a tendency for the capillary diameter to increase and the tissue pressure would thus be subject to an opposite force tending to balance the direct effect of the alveolar change. None of this takes into account forces within the alveolar wall membrane itself. Clearly the tissue pressure question cannot be resolved until an experimental approach is found.

The experiments of Welch[8] demonstrated that failure of the left ventricle to eject a full quota of blood could be associated with pulmonary edema. More recently, Paine *et al.* confirmed this.[18] Their method did not clearly exclude other reflexes, and some studies have suggested that reflexes are important in this experiment.[17] Cataldi[7] was able to produce pulmonary edema only rarely with even severe damage to the left ventricle. Consequently, it seemed reasonable to demonstrate that pulmonary capillary pressure could at least be raised to levels above the oncotic pressure by left ventricular failure.

Figure 2 shows the results of an experiment in which dogs were prepared beforehand by placing loose ligatures around the left anterior descending coronary artery and embedding the ligature ends under the skin of the chest wall for easy access later when, with an intact chest, occlusion of the coronary artery could be accomplished. An infusion of normal saline (85 ml./Kg./hr.) was administered. Two dogs received an infusion without occlusion, and two occlusion without infusion. Of the ten dogs who re-

ceived infusion and occlusion, the stroke volume rose by 20 per cent or more in four, and failed to rise or actually dropped in six. The pulmonary venous pressure rose very little in those dogs in which a rise in stroke volume was observed, but rose in some cases to extremely high levels in those in which the stroke volume did not rise when one could reasonably assume left ventricular failure. Probably coronary artery ligation injured the left

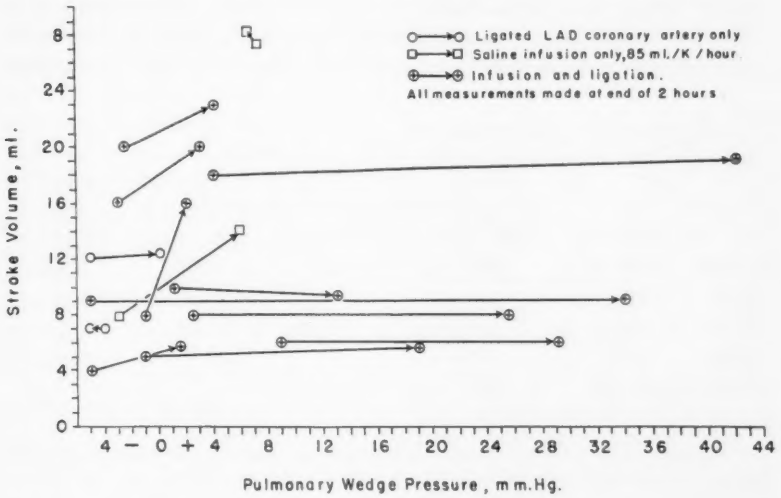


FIG. 2. Capillary pressure and stroke volume in experimental heart failure. The pressure rises significantly only when stroke volume cannot increase apace with load. Ligatures had previously been placed around the left anterior descending coronary artery with their ends embedded under the skin of the chest wall. They were pulled tight at the time the normal saline infusion (85 ml./K./hr.) was begun. Stroke volume and pressure studies were made one hour later.

ventricle in some dogs severely enough to preclude a normal response to increased filling pressure, whereupon, the pulmonary vascular pressure rose in proportion to the volume of fluid infused. This may not be quite analagous to the edema of heart failure in the absence of unusual saline loading of the circulation, but it does show that ventricular failure to meet a normally acceptable load rapidly leads to an elevated capillary pressure. Barring a change in capillary permeability or an incredible change in the rate of lymph flow, the accumulation of filtrate must have increased enormously in the above experiment. The animals did not survive long enough to develop clinically evident edema.

The importance of increased pulmonary capillary pressure in the genesis of pulmonary edema has been stressed.[18,19] It may be the most important single factor.[20] However, the mechanism of this elevation and protective reflexes are variable and controversial.

Pressure in the pulmonary circulation can be raised by experimentally induced left ventricular failure as already indicated, or by obstruction at the mitral valve.[21] The question of an active increase in resistance at the pulmonary venous level is unsettled, but sound evidence is available that a "throttle" mechanism at the level of the pulmonary veins is possible.[22,23] There appears to be no doubt that pulmonary venules in dogs[24] and guinea

TABLE 1. EFFECT OF NOR-EPINEPHRINE

Dog no.	*Lung wt./ body wt.**	*Pressure change,** mm. Hg*			*Cardiac output change, per cent*
		Femoral artery	*Pulmonary artery*	*Pulmonary wedge*	
58-28	1.535	+66/40	0	0	+11
58-29	1.320	+121/100	+27/34	+3	—12
59-02	1.275	+88/32	+36/0	—1	—20
59-03	1.180	+38/25	+29/16	+0.5	—11

* Expressed as fresh wet weight, grams/100 grams body weight.
** Pressures were observed 15 minutes and cardiac outputs 18-20 minutes after the injection of Levophed base (0.03 mg./K). Dogs were sacrificed one hour after injection.

pigs, rabbits, and cats[25-27] are capable of active constriction.

Experimental pulmonary edema produced by adrenalin has been demonstrated for over half a century[28-30] and it has been known to produce pulmonary edema in guinea pigs,[31] rabbits,[32,33] cats,[34,35] and dogs.[36] The precise effect on the filtration pressure is uncertain, however, since it appears capable, in the doses used, of producing left ventricular failure[36] or at least raising the left atrial pressure,[34] suggesting a secondary cause for high filtration pressure. Some studies have suggested a constricting effect on pulmonary arterioles because of the high pulmonary artery pressure reported.[37-39] Failure of the right heart relative to adrenalin was suggested by the work of Elkeles[32] in which diodrast, injected into a peripheral vein, failed to enter the right atrium after adrenalin pulmonary edema.

In a study of the effect of nor-epinephrine on dogs, we obtained results suggesting that pulmonary arteriolar constriction usually, but not always, prevents a rise in capillary pressure and prevents edema (Table 1).

Femoral and pulmonary arterial and wedge pressures were recorded before and after the intravenous injection of 0.40 ml. (40 μg. of base) of levarterenol bitartrate per kilogram. Only the first dog had an increase in lung-weight to body-weight ratio great enough to suggest pulmonary edema; this dog had no change in pulmonary arterial or wedge pressure, although a rise in femoral artery pressure was observed.

All of the other dogs had great increases in pulmonary artery pressure with little change in pulmonary wedge pressure, suggesting that an increase in pulmonary arterial resistance protected the capillaries against excessive filtration pressure. However, the evidence of edema in the first dog in the absence of a rise in pulmonary wedge pressure is puzzling. Since norepinephrine generally has little effect on blood flow, these data are, for the most part, the reflection of pressure phenomena, and the effect is probably exerted on arteriolar resistance. Whether high pressure in the pulmonary capillaries can lead to reflex increase in arteriolar resistance is not known conclusively. It has already been pointed out that high pulmonary vascular pressure decreases resistance.[13-14, 40] This is probably a passive phenomenon, but increased capillary or venous pressure might reflexly increase arteriolar resistance. Former studies from our laboratory suggested such reflexes.[41]

In another group of experiments the pulmonary veins draining the isolated left lower lobe of a dog's lung were ligated, the remaining vessels being patent, and the pressure in the right branch pulmonary artery noted. Figure 3 shows the results of such an experiment in seven dogs in which the cardiac output did not change significantly. The right pulmonary arterial pressure rose within minutes after ligation of the left lower lobe pulmonary vein in spite of the fact that only a small part of the pulmonary circulation was obstructed. When the left lower lobe pulmonary artery was ligated, no change was noted in the right branch pulmonary arterial pressure; thus, the pressure rise does not seem to be the result of the extra volume flow shunted into the right lung. Ligation of the left lower lobe pulmonary vein must have produced an increase in vascular resistance in the right lung which did not occur when the left lower lobe pulmonary artery was ligated. Von Euler and Liljestrand[35] found a slight rise in pulmonary arterial pressure when the contralateral pulmonary artery was clamped but this rise, also observed by Carlens *et al.*[42] was not significant.

Sanger *et al.*[43] demonstrated clearly that this effect is mediated through the autonomic nervous system when they observed an increased pulmonary arteriolar resistance on infusing saline into the left pulmonary veins. The rise was not found after section of the branches of the thoracic vagus and

bilateral thoracic sympathectomy. The sensitivity of the reflex is attested to by the experiment represented in Figure 3 in which merely occlusion alone without infusion produced the change. Ferguson and Berkas showed that edema could be produced when vascular pressures were raised by a systemic-pulmonary artery shunt with greater frequency after denervation.[44] On the other hand, Rodbard[45] has suggested that bronchomotor tone rather than arteriolar constriction governs pulmonary vascular resistance, but there is little direct evidence bearing on this point of view.

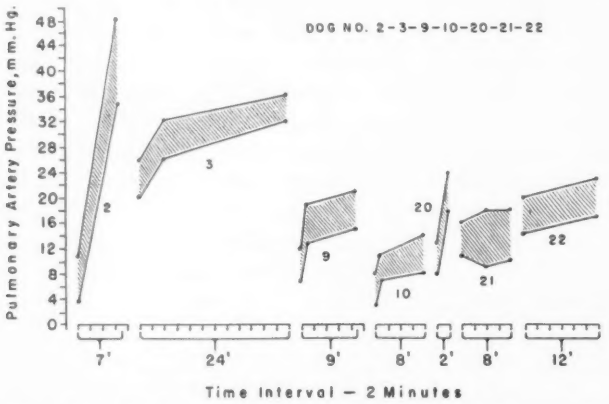


FIG. 3. The effect of unilobar pulmonary venous ligation on the contralateral pulmonary arterial pressure. The cardiac output did not change significantly in this study. The method has been described in an earlier report.[43]

Increased intracranial pressure,[46,47] injection of fibrin into the cisterna,[48-50] and large rapid carotid artery infusion[51] have produced rises in pulmonary wedge or left atrial pressures often with pulmonary edema formation. Bradycardia usually accompanied the pulmonary edema, and atropine may protect against this type of pulmonary edema.[47]

In one study experimental pulmonary edema occurred at left atrial pressures ranging from 30 to 90 mm. Hg, but failed to occur in other experiments with left atrial pressures ranging from 13 to 45 mm. Hg.[52] This lack of correlation between pressure and edema has been noted by others.[4] One might expect that pulmonary capillary pressure could be raised by left heart failure, increased resistance to blood flow through the pulmonary veins or left heart, or by reflexes causing peripheral vasoconstriction with a shift of blood into the lungs. In some cases, reflex constriction of the pulmonary veins and shifting of blood into the lungs may be

concomitant. Furthermore, once the pulmonary vascular pressure is raised, other phenomena may encourage or prevent edema formation. Pulmonary edema can be prevented by stellate ganglionic block,[80] reducing venous return by phlebotomy, and ganglionic blocking agents producing peripheral pooling of blood.[80]

Some of these regulating factors may influence the blood and tissue fluid osmotic pressure, capillary permeability, or lymph flow. The net osmotic pressure is not known precisely, since the osmotic pressure of tissue fluid is unknown. The latter is probably lower than that of lymph[19] and certainly lower than blood. Blood has an effective colloid osmotic pressure of about 25 mm. Hg, the net osmotic pressure being the difference between this and the tissue fluid osmotic pressure. Whatever the net difference may be, it does not seem likely that gross changes occur unless there are marked changes in capillary permeability. Crystalloids are usually thought to be freely diffusible through the capillary membrane, although studies with urea have cast doubt on this.[58] Proteins are not ordinarily diffusible, hence, changes in permeability to proteins could quickly alter the net osmotic pressure.

Studies of the fluid in pulmonary edema have suggested a protein concentration of up to three or four per cent[3] or more,[8] which is higher than in the case of the peripheral edema of heart failure. This suggests increased permeability in some cases but doesn't tell us whether the increased permeability is the result of initiating factors such as hypoxia, of reflexes, or simply of the high pulmonary capillary pressure exerted against the capillary walls. Greater capillary permeability may be part of the precipitating mechanism, and in turn be perpetuated by the hypoxia and high transmural pressure of pulmonary edema. Drinker has maintained that hypoxia is an important initiating event in the permeability change,[18] but the concept rests on indirect evidence.

Filtration of water and filterable substances is related to the physical character of the capillary membrane, i.e., thickness, structure, etc., and the filtration pressure. The latter is the resultant of the forces across the membrane, and the former may be expressed as a filtration coefficient:

$$F = Kp\ [(pC - pT) - (\pi c - \pi t)],$$

where F = net transfer of fluid; Kp = filtration coefficient; pC = capillary hydrostatic pressure; pT = tissue hydrostatic pressure; πc = micron blood osmotic effect; πt = tissue fluid osmotic effect.[58]

When the net transmural pressure is higher than the net osmotic pressure, F is positive and flow is out of the capillary. When the reverse holds, flow is into the capillary. Kp in the hind limb capillaries of a cat has been

calculated at 3.4 x 10^{-4} cu.μ./sec./mm.Hg./sq.μ.[53-55] Assuming some similarity to the Kp for the cat's lung capillaries, and assuming a 2 kilogram cat with a lung capillary membrane surface of 4 sq. m., a net flux pressure, [(pC — pT) — (πc — πt)], of 1 mm. Hg would produce a net outward flow of 5 ml./hour (see Fig. 4). This is roughly of the same order as right lymph duct flow measurements.[21, 56, 57] When the transmural pressure

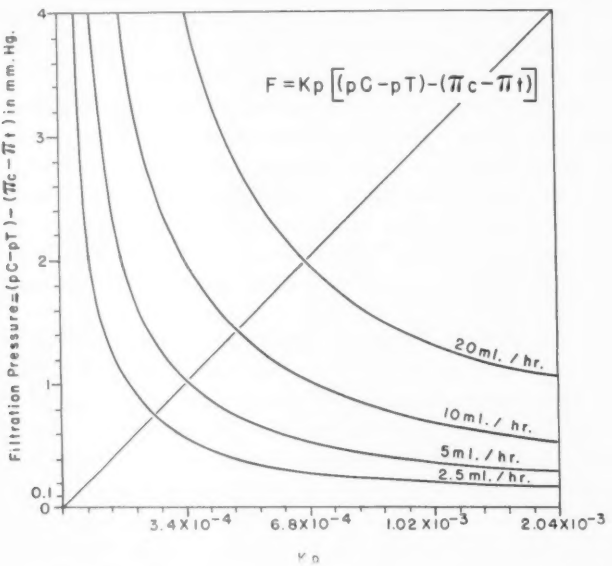


FIG. 4. The relationship of filtration rate to the filtration coefficient and pressure. At high filtration pressures the coefficient of filtration has the greater effect on filtration rate, conversely at low pressures the filtration pressure has a greater effect.

difference is low and Kp high, pressure change has a great effect on flux, but when the reverse holds, Kp has a greater influence (see Fig. 4). Prolonged elevation of capillary pressure probably causes endothelial thickening which would lower Kp, thus shifting F back to a lower flow rate.

Similarly, increased intracapillary pressure might open wider the capillary pores or interstices through which protein molecules could escape. This would reduce the effective colloid osmotic pressure within the capillary, thus enhancing loss of fluid. In short, one can expect changes correlated with rises in hydrostatic pressure, but these changes are variable and might, in some cases, actually work in antagonistic directions.

The role of the capillary membrane is poorly defined experimentally, but in theory it is important. Drinker[18] attached great significance to the influence of hypoxia on capillary permeability, and there is evidence that permeability increases in anemia with or without congestive heart failure.[58] Temperature appears to be inversely related to the filtration coefficient.[59] Stretching of the capillary wall has been implicated as a cause for increased permeability by widening pore size, but no direct experimental evidence exists in regard to lung capillaries. Cameron and De[48] suggested direct vagal influence on capillary permeability.

Increasing the total intravascular volume of water and crystalloid will increase the filtration because simple diffusion would increase the amount of fluid on both sides of the capillary wall even if there were no osmotic effect; but in addition, the semi-permeable character of the membrane is such that the initial result of a water load is to lower the intracapillary osmotic pressure, thus diluting proteins and encouraging outward filtration until a new balance of forces is established. This may take as long as an hour.[54]

The maximum lymph drainage, which may be up to 60 ml./hour in dogs,[21] is such that animals with lymphatic obstruction develop edema over a period of hours. This would not explain the precipitous occurrence of edema seen in clinical acute pulmonary edema. Furthermore, there may be no lymphatics around the alveoli themselves[39]; hence the greatest area of transudation in lung edema is not directly affected by lymph drainage.

The possibility that there are changes in the electrolyte and water content of blood which occur as it traverses the capillary bed, in the course of pulmonary edema, has led to a series of experiments summarized in Table 2. Edema was produced by injecting starch according to the method of Singer *et al.*,[60] or alpha naphthylthiourea (ANTU) as was done by Drinker,[18] Latta,[61] Richter,[62] and Williams.[63] During the period of the experiment (about one hour), the hemoglobin level was little altered by the procedure, nor was there a great change in electrolyte concentration or pH across the lung capillaries (Table 2). In the case of the ANTU experiments, crystalloid osmotic pressure rose after the injection but no transcapillary change occurred. In the case of starch experiments, pulmonary arterial pressure rose after the injection, probably as a result of embolism plus reflex effects on the pulmonary arterioles.[60, 64] Fluid loss from capillary transudation in pulmonary edema must lead to hemoconcentration, but the process appears to be so gradual that it is not easily measured. The consequent change in the physical character of the blood must be too slight to be of importance as a cause of further edema.

The intense cyanosis associated with severe edema of the lung testifies to the profound impairment of gas exchange. Edema of the pulmonary capillaries and airway passages usually does not interfere significantly with carbon dioxide exchange; however, oxygen uptake may be seriously impaired. This suggests that venous admixture, a capillary block due to

TABLE 2. CHANGES IN BLOOD CONSTITUTION FROM PULMONARY ARTERY TO PULMONARY VEIN IN THE COURSE OF EXPERIMENTAL EDEMA

	*Change**							
	Hemato-crit %	*Hemo-globin, G.*	*Osmo-larity, mM/L*	*Sodium mE/L*	*Chloride mE/L*	*CO_2 mM/L*	*pH*	*Pressure mm. Hg*
	7 ANTU experiments—lung wt./body wt. over 1.4							
PA,** after-before	0	0	+31.3	0	0	—2.9	0	0
FA†-PA before	0	0	0	0	0	—2.6	0	—
FA-PA after	0	0	0	—2.7	+3.6	—3.5	0	—
Pulm. wedge	—	—	—	—	—	—	—	0
	8 starch experiments—lung wt./body wt. over 1.4							
PA, after-before	0	0	0	—9.6	—4.9	—1.25	0	+13.7
FA-PA before	0	0	0	0	0	—1.28	0	—
FA-PA after	0	0	0	0	0	—1.99	0	—
Pulm. wedge	—	—	—	—	—	—	—	0
	10 starch experiments—lung wt./body wt. less than 1.4							
PA, after-before	0	0	0	0	0	—1.0	0	+18.4
FA-PA before	0	0	0	0	0	—2.2	0	—
FA-PA after	—1.8	0	0	0	+5.1	—2.2	0	—
Plum. wedge	—	—	—	—	—	—	—	+4.6

* When the "p" value was greater than 0.05, the change was recorded as zero. The ANTU experiments were performed according to the technique used by Williams,[88] and the starch experiments followed the procedure of Singer *et al.*[80]

** P.A. = Pulmonary artery.

† F.A. = Femoral artery.

thickened membrane, and decreased capillary diffusing surface are the causes of the hypoxia, for if the trouble were ventilatory, carbon dioxide accumulation would occur. However, the possible role of bronchial obstruction or increased rigidity of the lung in edema has not been fully evaluated.

In acute experiments in which dogs were subjected to rapid infusion after ligation of the left anterior descending coronary artery, edema of the lung occurred. The degree of arterial oxygen saturation deficit appeared to

be correlated with the pulmonary venous pressure (Fig. 5). A gradual decline of oxygen saturation occurred as the pressure rose. At very high pulmonary venous pressures the saturation was extremely low. The same relationship was found between the pulmonary wedge pressure and the arterial oxygen saturation in a group of patients with mitral stenosis (Fig. 6), except that the data could be grouped equally well about two additional slopes; the first relating pulmonary wedge pressure and oxygen saturation in a range of 4 to 28 mm.Hg, the second with a similar angle of slope but

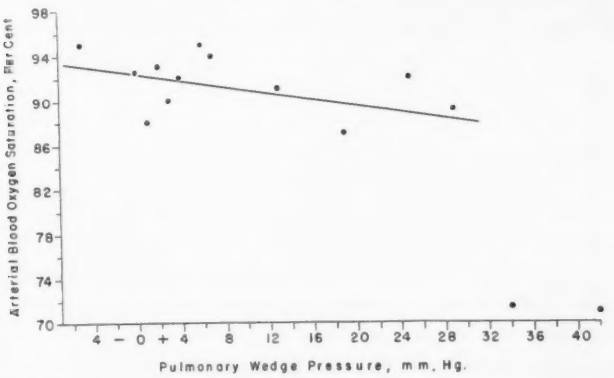


FIG. 5. Relationship between pulmonary artery wedge pressure and arterial oxyhemoglobin saturation during experimental left ventricular failure in dogs. The experimental technique has been described in the legend for Figure 2.

a higher level for a pressure range of 26 to 45 mm.Hg. Compensatory adjustments may lead to higher arterial oxygen saturations at a critical pulmonary capillary pressure level. The physiological dead space might be diminished with subsequent improvement of arterial oxygen saturation if perfusion channels were opened in hitherto poorly perfused but well ventilated areas of lung, or an increase in diffusing capacity might occur. Distention of the capillaries under high pressure could increase the diffusing capacity by increasing the capillary diffusing surface area, although such an effect is not easily demonstrated by current methods.[85] Hyperventilation may increase the diffusing capacity,[86] and in addition, it raises the alveolar oxygen tension. These results of hyperventilation are quite likely to raise the slope relating oxygen saturation to capillary pressure at higher capillary pressure levels. Hyperventilation might be mediated through baroreceptors in pulmonary vessels or the Hering-Breuer reflexes related to increased intrathoracic pressure fluctuations brought about by

bronchial obstruction. Alveolar ventilation and diffusion studies were not done on the patients represented in Figure 6.

Alternatively, prolonged exposure of the capillary wall to high pressure might alter its physical characteristics and its filtration coefficient. The importance of a decrease in the latter at high pressure is shown in Figure 4. Thus, a patient with very high pulmonary capillary pressure existing over a long period of time might have less edema than one with less elevation

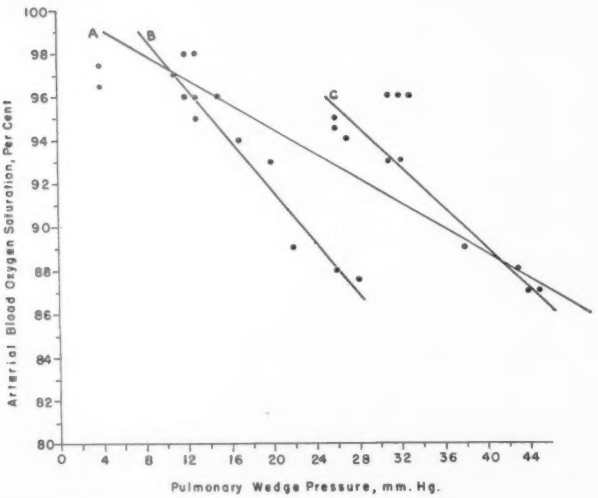


FIG. 6. Relationship between pulmonary artery wedge pressure and arterial oxyhemoglobin saturation in patients with mitral stenosis. Neither pressures nor oxygen saturations could be correlated with duration or severity of symptoms. Slope A ($r = -0.62$, standard error 0.2) represents the general correlation of all data; slope B ($r = -0.87$, standard error 0.3) representing the lower wedge pressures can be distinguished from slope C ($r = -0.73$, standard error 0.3) representing the higher wedge pressures.

for a shorter time. This would be consistent with reports in which the pulmonary capillary pressure and clinical edema were not uniformly related in patients with mitral stenosis.[67, 68]

Christie[60] and others[41, 70] have presented evidence that pulmonary engorgement may lower lung compliance. Bronchial obstruction and a change in surface tension of the alveolar walls, however, may contribute to the greater work of ventilating an edematous lung.[71, 72] To apportion quantitative values to these factors is probably beyond the ability of current research techniques.

A large part of the hypoxia observed in pulmonary edema may be due to

a simple fluid block at the capillary level. This has been confirmed by observing hypoxia in patients with pulmonary edema in the presence of normal arterial carbon dioxide tensions. When experimental pulmonary edema is produced in dogs, the alveolar arterial oxygen pressure gradient rises sharply. Changes in the carbon monoxide diffusing capacity are less consistent, but in general it does not change when the pulmonary capillary pressure rises.[65] The significance of the latter is clouded by the fact that little is known of what D_{co} actually measures. Further studies are needed, but these data suggest a venous admixture. Auld *et al.*[73] have found evidence that elevated pulmonary arterial or left atrial pressures may reverse blood flow through bronchial-to-pulmonary artery collateral channels, thus producing a venous admixture.

CONCLUSIONS

Virtually the same volume of blood perfuses the lung capillaries as all of the other organs and tissues of the body combined. The net pulmonary capillary pressure is ordinarily low, but any appreciable increase in the volume of perfusion of the capillaries mediated through circulatory reflexes, or any increase in the resistance to flow beyond the capillary bed will raise the net capillary hydrostatic pressure unless the distensibility of the capillaries is high, or mechanical factors and vasomotor reflexes come into play to protect the capillary bed. Referring to Figure 1, it becomes apparent that there is room for almost unlimited expansion of capillary volume, the limiting factor being the distensibility of the capillary wall itself. Even this could be ameliorated by opening new capillary channels or new pathways of venous drainage. The latter might occur if, for example, in the presence of pulmonary venous hypertension due to left heart failure, anastomoses between pulmonary and bronchial veins (Figure 1) were to increase. The most direct mechanisms for preventing rises in pulmonary capillary pressure would be neurogenic constriction of the pulmonary arteries or an increase in intrapulmonary pressure due to airway obstruction because of bronchial mucosal edema or bronchospasm. The latter would reduce venous return.

There is ample evidence that net hydrostatic pressure may rise to edema levels, but there is also evidence that the factors leading to pulmonary capillary hypertension may be frustrated by protective reflexes or that, even though the pressure rises, changes in membrane permeability, tissue pressure, or lymph drainage can deter the development of edema. Although electrolyte and colloid osmotic pressure phenomena are very important, the evidence that significant changes occur in association with pulmonary edema is not conclusive at present.

Capillary membrane permeability remains one of the unknown factors of pulmonary transudation, although the anatomical position of the membrane suggests a key role. The membrane is endowed with a measureable filtration coefficient which is subject to the effect of physical and chemical influences, and probably nervous and hormonal control of this filtration coefficient plays a part in edema formation.

Lymph drainage is an obvious factor in capillary fluid exchange but present indications are that it is much more important in the slow, gradual edema accumulation of chronic passive congestion than in the rapidly extensive flooding of the lung characteristic of acute pulmonary edema.

In chronic passive congestion of the lung, alveolar edema and transudation in the visceral pleura probably proceed together, and when right-sided heart failure supervenes, the enormous transuding surface of the parietal pleura (perfused by systemic capillaries) contribute to massive pleural effusion (see Fig. 1). It is difficult to see why pleural effusion is not more common in acute pulmonary edema. The intrapleural pressure is more negative in relation to the atmosphere than the intra-alveolar pressure, hence with acute increases in capillary pressure, there should be a great outflow of fluid from the visceral pleura. Possibly the lymphatics in the pleura protect it in contrast to the alveoli where no lymphatics are present, or possibly in acute pulmonary edema, reflexes impair the permeability of the alveolar-capillary membrane but not the pleural capillary wall.

In short, all factors of the current hypotheses of capillary fluid interchange are concerned in edema of the lung, but their relative importance varies depending upon the circumstances leading to the edema, and no one factor can be considered the *sine qua non* of lung edema. The clinical character of the edema is more dependent on the cause and factors leading up to alteration of the capillary fluid interchange than it is on local phenomena. Thus, acute pulmonary edema is a rapid neurogenic phenomenon with a receptor organ, afferent pathways, central connections, and efferent pathways. It includes effector and suppressor systems. Chronic passive congestion is a more gradual response to left heart failure.

At the capillary level, edema accumulation, regardless of the cause or initiating pattern, is related to a change in the balance of net hydrostatic and osmotic pressures, membrane diffusion and lymphatic drainage, and the volume of circulating water and electrolytes.

SUMMARY

A review of the local phenomena of capillary fluid interchange in edema of the lung indicates that all of the known factors of transudation are impli-

cated in edema of the lung, but with varying degrees of importance depending upon the circumstances producing the imbalance. Acute pulmonary edema differs from chronic passive congestion largely because of the neurogenic pattern of the former, and the exclusive role of left heart failure in the latter. At the capillary level, both involve the same principles of fluid interchange.

REFERENCES

1. Albertini, F. I.: De Affectionibus Cordis (1726), quoted by Willius, F. A. and Dry, T. J.: *A History of the Heart and Circulation.* Philadelphia, W. B. Saunders, 1948.
2. Altschule, M. D.: *Acute Pulmonary Edema.* New York, Grune and Stratton, 1954.
3. Luisada, A. A. and Cardi, L.: Acute pulmonary edema. Pathology, physiology, and clinical management. *Circulation,* 1956, *13,* 113-135.
4. Shedd, D. P. and Gray, F. D., Jr.: Experimental unilobar pulmonary edema. *Yale J. Biol. Med.,* 1957, *30,* 110-120.
5. Welch, W. H.: Zur Pathologie des Lungenödems. *Virchow Arch. f. path. anat. u. physiol.,* 1878, *72,* 375-412.
6. Henneman, P. H.: Acute pulmonary edema, with special reference to experimental studies. *New Engl. J. Med.,* 1946, *235,* 590-596 and 619-624.
7. Cataldi, G. M.: Oedème aigu du poumon dans les lesions expèrimentales du ventricule droit. *Arch. des Maladies du Coeur, des Vaisseaux et du sang.* 1935, *28,* 604-608.
8. Cameron, G. R.: Pulmonary oedema. *Brit. med. J.,* 1948, *1,* 965-972.
9. Cudkowicz, L., Calabresi, M., Nims, R. G., and Gray, F. D., Jr.: The simultaneous estimation of right and left ventricular outputs applied to a study of the bronchial circulation in dogs. *Amer. Heart J.,* 1959, *58,* 732-742.
10. Comroe, J. H., Jr., Forster, R. E., II, Dubois, A. B., Briscoe, W. A., and Carlsen, E.: *The Lung,* Chicago, The Year Book Publishers, 1955.
11. Green, H. D.: Circulation: Physical principles in *Medical Physics.* Volume I, 208-232, Chicago, The Year Book Publishers, 1944.
12. Carlill, S. D. and Duke, H. N.: Pulmonary vascular changes in response to variations in left auricular pressure. *J. Physiol.,* 1956, *133,* 275-286.
13. Lendrum, B. L. and Lichtman, A. M.: Pulmonary vascular resistance in man at different intravascular distending pressures measured in a case of mitral stenosis complicated by anomalous pulmonary venous connection. *Circulation,* 1957, *16,* 1090-1096.
14. Williams, M. H., Jr.: Relationships between pulmonary artery pressure and blood flow in the dog lung. *Amer. J. Physiol.,* 1954, *179,* 243-245.
15. Lloyd, T. C., Jr. and Wright, G. W.: Pulmonary vascular resistance and vascular transmural gradient. *J. appl. Physiol.,* 1960, *15,* 241-245.
16. Paine, R., Butcher, H. R., Smith, J. R., and Howard, F. A.: Observations on the role of pulmonary congestion in the production of edema of the lungs. *J. Lab. and clin. Med.,* 1950, *36,* 288-296.
17. Teisser, J.: De l'oedème aigu du poumon. *C. R. 13th Congrès International de Médecins, Paris 1900.* Cited by Henneman (6).
18. Drinker, C. K.: *Pulmonary Edema and Inflammation,* Cambridge, Mass., Harvard Univ. Press, 1947.
19. Visscher, M. B., Haddy, F. J., and Stephens, G.: The physiology and pharmacology of lung edema. *Pharmacol. Rev.,* 1956, *8,* 389-434.

20. Visscher, M. B.: The genesis of lung edema (Editorial). *Circulat. Res.*, 1954, *2*, 291-293.
21. Rabin, E. R. and Meyer, E. C.: Cardiopulmonary effects of pulmonary venous hypertension with specific reference to pulmonary lymphatic flow. *Circulat. Res.*, 1960, *8*, 324-335.
22. Aviado, D. M. and Schmidt, C.: Respiratory burns with special reference to pulmonary edema and congestion. *Circulation*, 1952, *6*, 666-680.
23. Burch, G. E. and Romney, R. B.: Functional anatomy and "throttle valve" action of the pulmonary veins. *Amer. Heart J.*, 1954, *47*, 58-66.
24. Aviado, D. M.: Pulmonary venular response to anoxia, 5-hydroxytryptamine, and histamine. *Amer. J. Physiol.*, 1960, *198*, 1032-1036.
25. Irwin, J. W.: The pulmonary microcirculation. *R. I. med. J.*, 1960, *43*, 522-527.
26. Nisell, H. O.: Reactions of the pulmonary venules of the cat with special reference to the effect of the pulmonary elastance. *Acta physiol. scand.*, 1951, *23*, 361-374.
27. Nisell, H. O.: Lungcirkulationens reglering. *Nord. med. Ark.*, 1951, *46*, 1560-1562.
28. Auer, I. and Gates, F. L.: Experiments on the causation and amelioration of adrenalin pulmonary edema. *J. exp. Med.*, 1917, *26*, 201-220.
29. Bouchard, C. and Claude, H.: Recherches expérimentales sur l'adrénaline. *C. R. Acad. Sci. (Paris)*, 1902, *135*, 928-931.
30. Luisada, A. A.: The pathogenesis of paroxysmal pulmonary edema. *Medicine*, 1940, *19*, 475-504.
31. MacKay, E. M. and Peska, E. F.: Studies of experimental pulmonary edema. I. Pulmonary edema from l-epinephrine and l-norepinephrine (arterenol). *Proc. Soc. exp. Biol. (N. Y.)*, 1949, *71*, 669-670.
32. Elkeles, A.: Angiocardiographic experiments on the pathogenesis of adrenalin pulmonary edema in rabbits. *Brit. J. Radiol.*, 1948, *21*, 472-476.
33. Stone, C. H. and Loew, E. R.: Effect of various drugs on epinephrine-induced pulmonary edema in rabbits. *Proc. Soc. exp. Biol. (N. Y.)*, 1949, *71*, 122-126.
34. Bucher, K. and Hürlimann, A.: Über die Bedeutung von Druck und Blutangebot in den Vorhöfen für die Hämodynamik von Lungen—und Korperkreislauf. *Helv. physiol. pharmacol. Acta*, 1950, *8*, 317-330.
35. v. Euler, U. S. and Lilijestrand, G.: Observations on the pulmonary arterial blood pressure in the cat. Acta physiol. scand., 1946, *12*, 301-320.
36. Paine, R., Smith, J. R., Butcher, H. B., and Howard, F. A.: Heart failure and pulmonary edema produced by certain neurologic stimuli. *Circulation*, 1952, *5*, 759-765.
37. Born, G. V. R.: Acute oedema in the isolated perfused lungs of rabbits. *J. Physiol.*, 1954, *124*, 502-514.
38. Daly, I. deBurgh: Reactions of the pulmonary and bronchial blood vessels. *Physiol. Rev.*, 1933, *13*, 149-184.
39. Edwards, W. S.: The effects of lung inflation and epinephrine on pulmonary vascular resistance. *Amer. J. Physiol.*, 1951, *167*, 756-762.
40. Borst, H. G., McGregor, M., Whittenberger, J. L., and Berglund, E.: Influence of pulmonary arterial and left atrial pressures on pulmonary vascular resistance. *Circulat. Res.*, 1956, *4*, 393-399.
41. Gray, F. D., Jr., Shedd, D. P., and Kato, E.: Respiratory responses to experimental unilobar pulmonary edema. *Circulat. Res.*, 1958, *6*, 507-515.
42. Carlens, E., Hanson, H. E., and Nordenström, B.: Temporary unilateral occlusion of the pulmonary artery, a new method of determining separate lung function and of radiologic examinations. *J. thorac. Surg.*, 1951, *22*, 527-539.
43. Sanger, P. W., Robicsek, F., Taylor, F. H., Magistro, R., and Foti, E.: Observations on pulmonary vasomotor reflexes. *J. Thorac. Sur.*, 1959, *37*, 774-782.

44. Ferguson, D. J. and Berkas, E. M.: Effect of lung denervation on pulmonary hypertension and edema. *Circulat. Res.,* 1957, *5,* 310-314.

45. Rodbard, S.: Bronchomotor tone. A neglected factor in the regulation of the pulmonary circulation. *Amer. J. Med.,* 1953, *15,* 356-367.

46. Campbell, G. S., Haddy, F. J., Adams, W. L., and Visscher, M. B.: Circulatory changes and pulmonary lesions in dogs following increased intracranial pressure and the effect of atropine upon such changes. *Amer. J. Physiol.,* 1949, *158,* 96-102.

47. Harrison, W. and Liebow, A. A.: The effects of increased intracranial pressure on the pulmonary circulation in relation to pulmonary edema. *Circulation,* 1952, *5,* 824-832.

48. Cameron, G. R. and De, S. N.: Experimental pulmonary oedema of nervous origin. 1949, *61,* 375-387.

49. Sarnoff, S. S. and Berglund, E.: Neurohemodynamics of pulmonary edema. IV. Effect of systemic vasoconstriction and subsequent vasodilation on flow and pressure in systemic and pulmonary vascular beds. *Amer. J. Physiol.,* 1952, *170,* 588-600.

50. Sarnoff, S. S. and Sarnoff, L. C.: Neurohemodynamics of pulmonary edema. I. Autonomic influence on pulmonary vascular pressures and the acute pulmonary edema state. *Dis. Chest,* 1952, *22,* 685-698.

51. Luisada, A. A. and Contro, S.: Experimental pulmonary edema following rapid carotid infusion: mechanism and therapy. *Circulat. Res.,* 1953, *1,* 179-183.

52. Cross, C. E., Rieben, P. A., and Salisbury, P. F.: Urea permeability of alveolar membrane; hemodynamic effect of liquid in the alveolar space. *Amer. J. Physiol.,* 1960, *198,* 1029-1031.

53. Renkin, E. M.: In *Handbook of Circulation,* Section 23, p. 46, Philadelphia, W. B. Saunders Co., 1959.

54. Pappenheimer, J. R., Renkin, E. M., and Borrero, L. M.: Filtration, diffusion and molecular sieving through peripheral capillary membranes. A contribution to the pore theory of capillary permeability. *Amer. J. Physiol.,* 1951, *167,* 13-46.

55. Pappenheimer, J. R. and Soto-Rivera, A.: Effective osmotic pressure of the plasma proteins and other quantities associated with the capillary circulation in the hind-limbs of cats and dogs. *Amer. J. Physiol.,* 1948, *152,* 471-491.

56. Cameron, G. R. and Courtice, F. C.: The production and removal of oedema fluid in the lung after exposure to carbonyl chloride (phosgene). *J. Physiol.,* 1946, *105,* 175-185.

57. Warren, M. F., Peterson, D. K., and Drinker, C. K.: The effects of heightened negative pressure in the chest together with further experiments upon anoxia in increasing the flow of lung lymph. *Amer. J. Physiol.,* 1942, *137,* 641-648.

58. Sanghvi, L. M. and Wintrobe, M. M.: In *Handbook of Circulation,* Section 135, p. 349, Philadelphia, W. B. Saunders Co., 1959.

59. Miller, W. S.: *The Lung, (2d edition),* Charles C Thomas, Springfield Ill., 1947.

60. Singer, D., Hesser, C., Pick, R., and Katz, L.: Diffuse bilateral pulmonary edema associated with unilobar miliary pulmonary embolization in the dog. *Circulat. Res.,* 1958, *6,* 4-9.

61. Latta, H.: Pulmonary edema and pleural effusion produced by acute alpha-naphthyl thiourea poisoning in rats and dogs. *Bull. Johns Hopk. Hosp.,* 1947, *80,* 181-197.

62. Richter, C. P.: The physiology and cytology of pulmonary edema and pleural effusion produced in rats by alpha-naphthylthiourea. *J. thorac. Surg.,* 1952, *23,* 66-91.

63. Williams, M. H., Jr.: Effect of ANTU-induced pulmonary edema on the alveolar-arterial oxygen pressure gradient in dogs. *Amer. J. Physiol.,* 1953, *175,* 84-86.

64. Niden, A. H. and Aviado, D. M., Jr.: Effects of pulmonary embolism on the pulmonary circulation with special reference to arteriovenous shunts in the lung. *Circulat. Res.,* 1956, *4,* 67-73.

65. Weil, J.: Diffusing capacity in pulmonary congestion. *To be published.*

66. Filley, G. F., MacIntosh, D. J., and Wright, G. W.: Carbon monoxide uptake and pulmonary diffusing capacity in normal subjects at rest and during exercise. *J. clin. Invest.,* 1954, *33,* 530-539.

67. Ellis, L. B., Bloomfield, R. A., Graham, G. K., Greenberg, D. J., Hultgren, H. N., Kraus, H., Maresh, G., Mebane, J. G., Pfeiffer, P. H., Selverstone, L. A., and Taylor, J. A.: Studies in mitral stenosis. I. A correlation of physiologic and clinical findings. *Arch. intern. Med.,* 1951, *88,* 515-531.

68. Werkö, L., Ek, J., Bucht, H., and Eliasch, H.: Correlation between renal dynamics, cardiac output and right heart pressures in mitral valvular disease. *Scand. J. clin. lab. Invest.,* 1952, *4,* 15-23.

69. Christie, R. V. and Meakins, J. C.: The intrapleural pressure in congestive heart failure and its clinical significance. *J. clin. Invest.,* 1934, *13,* 323-345.

70. Saxton, G. A., Jr., Rabinowitz, M., Dexter, L., and Haynes, F.: The relationship of pulmonary compliance to pulmonary vascular pressures in patients with heart disease. *J. clin. Invest.,* 1956, *35,* 611-618.

71. Berglund, H., Lagerlöf, H., Werkö, L., and Bucht, H.: The relation between pulmonary capillary venous pressure, pulmonary blood volume, and vital capacity. *Trans. Ass. Amer. Phycns,* 1949, *62,* 124-133.

72. Haddy, F. J., Campbell, G. S., and Visscher, M. B.: Pulmonary vascular pressures in relation to edema production by airway resistance and plethora in dogs. *Amer. J. Physiol.,* 1950, *161,* 336-341.

73. Auld, P. A. M., Rudolph, A. M., and Golinko, R. J.: Factors affecting bronchial collateral flow in the dog. *Amer. J. Physiol.,* 1960, *198,* 1166-1170.

Articles are accepted for publication in *The Yale Journal of Biology and Medicine* on the basis of merit and suitability. The privilege of submitting articles is not limited to those associated with Yale University and contributions from others are cordially invited.

The Board of Editors

JACQUES BENOIT*

Department of Histophysiology,
Collège de France, Paris

OPTO-SEXUAL REFLEX IN THE DUCK: PHYSIOLOGICAL AND HISTOLOGICAL ASPECTS**

When I returned to Strasbourg from a holiday in 1933 I was greatly interested in a recent publication by Thomas Hume Bissonnette, the well-known zoologist of Trinity College, Hartford, Connecticut. Bissonnette had observed that light, the universal and common environmental factor, stimulated the development of the starling's gonads, and induced a very important increase in their volume. Red light was particularly effective in advancing sexual maturity by several months. I decided to repeat this amazing experiment with a larger bird, whose size would allow me to perform operative procedures and to analyze, from both anatomical and physiological points of view, the mechanism of this astonishing action of visible radiations upon the gonads. I chose the domestic duck, a large, quiet, good-tempered animal with a sexual cycle as accentuated as that of the so-called wild birds (Fig. 1).

Three years later I had the pleasure and opportunity to spend a full year in the Department of Anatomy at Yale University, as a Rockefeller fellow. I can hardly find words to express my gratitude and admiration to Professor Edgar Allen, then head of the Department for he welcomed me with a kindness I shall never forget. And let me tell you how much I appreciate the hospitality of all of you and how happy I am to spend a few days with you here after tweny-five years.

In New Haven from 1936 to 1937, I continued the work I had started on the duck, and presented my first results. Today I would like to summarize, in a very condensed way, the most important conclusions I could draw from later experiments, which I performed alone or with various collaborators, particularly with Dr. Assenmacher.

---

* Professeur au Collège de France.

** Presented as the Harry Burr Ferris Lecture 4 May 1961.

Received for publication 24 May 1961.

THE EYE AND ITS RESPECTIVE ROLES ON THE HYPOPHYSIS AND THE HYPOTHALAMUS IN THE PHOTO-SEXUAL REFLEX

Artificial light strongly stimulates the testes of immature ducks when given for 16 hours a day for three weeks.[1-4] Ducks of the Pekin and Rouen breeds were used in the experiments.

It is known that the *pituitary* gland is essential to both initiation and maintenance of the photo-sexual reflex. When it is removed after the animal has been submitted to stimulation by light, the testes rapidly decrease to a resting state.[5]

Some experiments had shown that local application of light was acting on the eye itself.[6-8] However, strong light stimulation induced testicular growth even after complete severance of the optic nerve or bilateral removal of the eyes[8,9,10] (Fig. 2). These observations suggested the existence of a second photosensitive center. In 1936[11] I could not determine whether this "deep" photoreceptor was located in the pituitary gland or in adjacent nervous structures; subsequent experimentation, however, enabled me to conclude that it was located within the *hypothalamus*.[12] Direct supply of light onto the hypothalamus, by means of a glass tube or a quartz rod introduced through the socket of the eye after removal of the orbital organs and tissues, strongly stimulated the genital glands (Fig. 3).

Other physiologists, studying the melanophorotropic function, had already approached this problem. In a remarkable series of experiments on the minnow, von Fritsch[13] observed that application of light to the diencephalon induced the fish to turn dark. This effect was caused by expansion of the melanophores. Scharrer[14] obtained similar results in studying the response to light of the same animal. After performing different surgical interventions on the brain, he came to the conclusion that the central photoreceptor involved could be some sensorial ependymal cells, or secretory cells of the preoptic nucleus. In 1935 Rodewald,[15] experimenting on *Rana temporaria,* showed that the stimulating action of light on the melanophoretic function of the hypophysis vanished after destruction of the hypothalamus.

In another experiment on the duck, we succeeded in directing a thin beam of parallel rays onto different levels of the brain surface. Direct supply of light onto the visual cortex itself was ineffective, whereas it induced a genital response when applied to the hypothalamus and to the rhinencephalon, a structure very closely connected to the diencephalic centers[13] (Fig. 4).

The evidence for the existence of both peripheral and central photoreceptors is summarized in the following experiment[13,14] (Fig. 5). Immature drakes were tied to specially devised wooden boards, groups of two animals

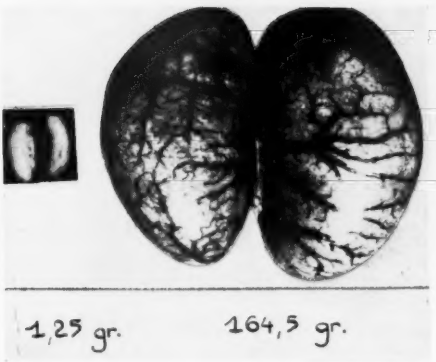
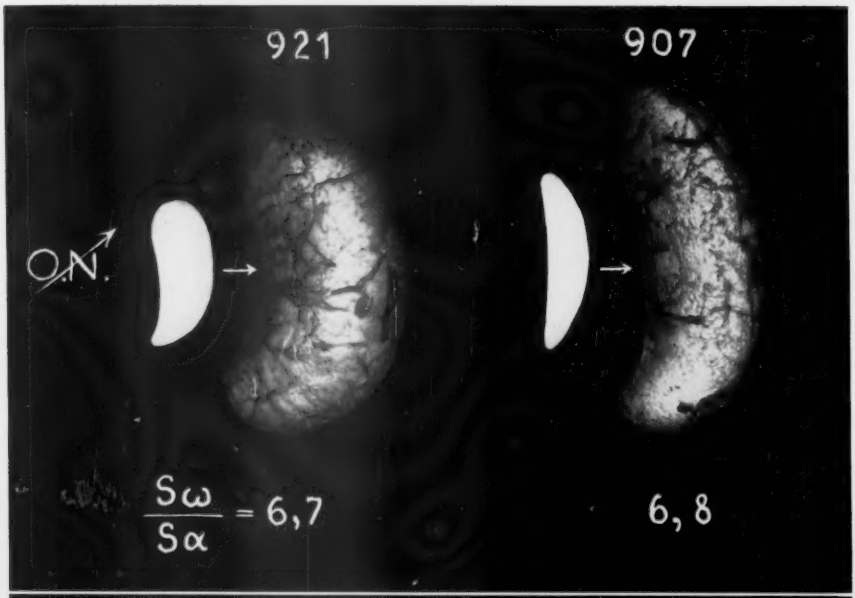


FIG. 1. Testes of adult Pekin ducks killed in December (weight: 1.25 gr.) and in May (weight: 164.5 gr).



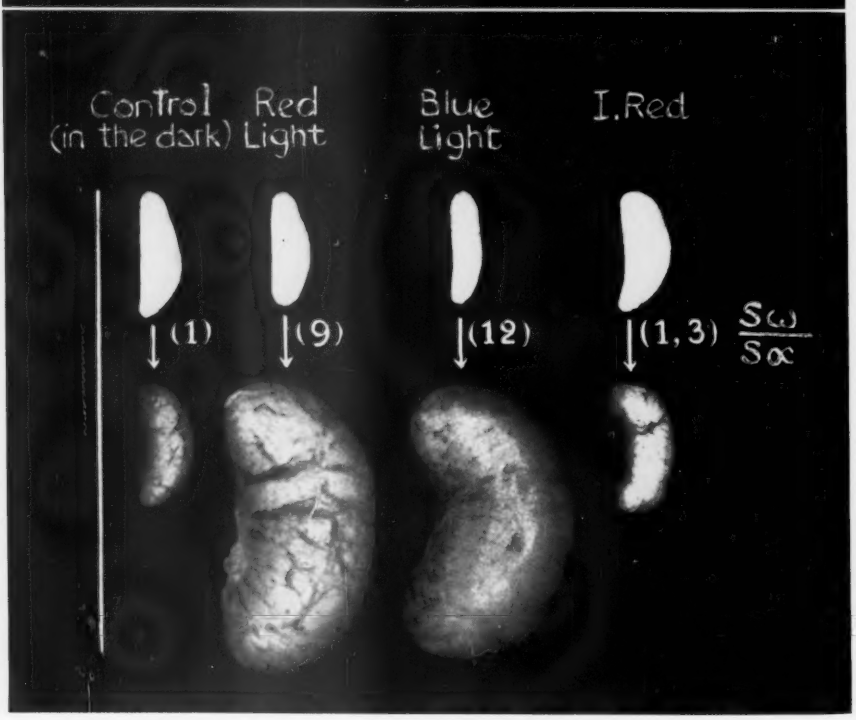


FIG. 2. Testes of two ducks given a fair amount of light during 16 hours a day after section of the optic nerves. Surface area was increased approximately 7 times.

FIG. 3. Surface increase ($S\omega/S\alpha$) of the left testis. In these subjects light was directly brought onto the hypothalamus through a quartz rod. Animal 3 received blue, animal 2 red, animal 4 infrared radiations. Subject 1 served as control; the quartz rod was introduced into the hypothalamus but no light was given. Compare the drawings representing the outline of the testes before the experiment with the photographs taken at autopsy.

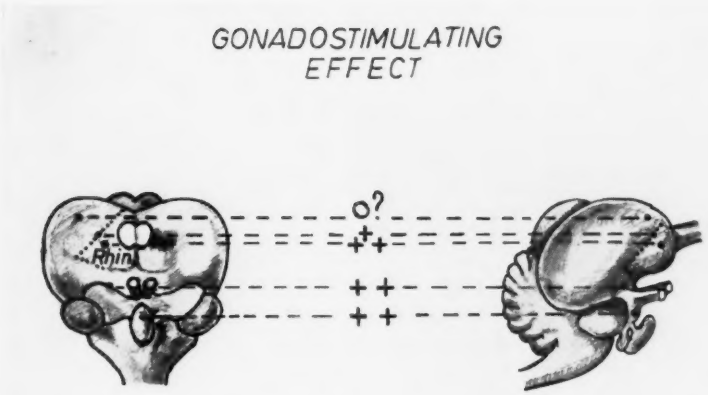


FIG. 4. Front view and side view of a duck's brain. Black dots represent the sites onto which parallel beams were directed. Treatment was applied three times for 22 hours at weekly intervals.

each placed at different distances from a weak source of light (240 lumen only). In each pair, one duck had one optic nerve sectioned, the contralateral eye being covered with a black screen; the second duck served as unoperated control. The ducks were then given artificial light for fifteen hours every fifth day during one month.

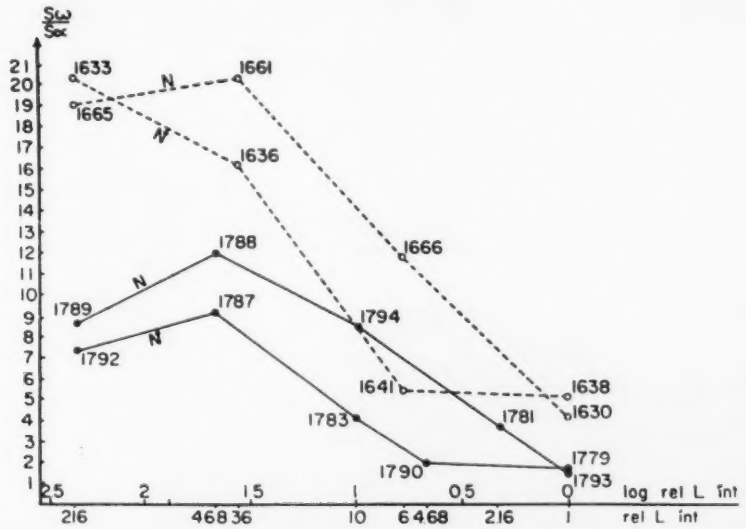


FIG. 5. Genital stimulation induced by light on normal ducks (N) and on ducks whose optic nerve has been sectioned on the illuminated side (N-with-arrow). The extent of the stimulation is proportional to the increase in testicular surface during the experiment (Sω/Sα). Normal and operated animals were paired and fixed at various distances from a source of light; their response is given in function of the relative light intensity. Operated ducks responded less than normal controls; lights stimulated both the superficial and deep receptors, in the N series, and the deep receptor only, in the N-with-arrow series.

At autopsy it was noted that: i) within a series, the testes grew the most when the distance to the source of light was the shortest; and ii), within each group the testes were less developed in the bird with section of one optic nerve. The action of light on only the central photoreceptor in the former case and on both the central and peripheral receptors in the latter account for this difference in testicular growth. The minor but positive testicular response in operated drakes can be accounted for by a penetration of light to the hypothalamus through the eye and the tissues of the orbital fossa.

Such penetration could be demonstrated by means of both photographic

and photoelectric methods.[15,18] The transmittance through the tissues is very weak for the short wavelengths (1/2000 for indigo radiations and 1/630 for green), but appears to be greater for longer wavelengths (1/130 for orange light and 1/55 for 647 mμ red radiations), so that red rays can penetrate to the hypothalamus with a sufficient intensity to activate the deeper photoreceptive structures, adding their effect to the action of the retina (Fig. 6). These observations suggest that visible radiations can

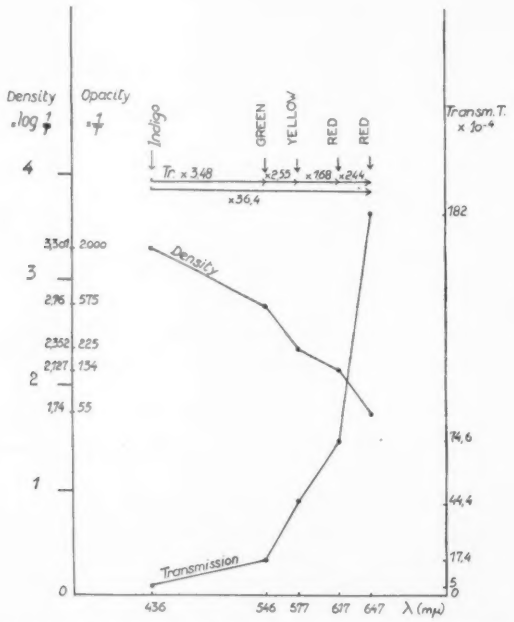


FIG. 6. Penetration of light to the hypothalamus across the eye and the orbital tissues. Note that transmission varies according to the wavelength; it increases from 1/2000 for indigo to 1/55 for red light ($\lambda = 647$ mμ).

stimulate the gonadotropic function of the adenohypophysis through both an oculo-hypothalamic pathway and a direct action on the hypothalmus across the orbital tissues.

MECHANISM OF THE HYPOTHALAMIC CONTROL OF THE PITUITARY GONADOTROPIC FUNCTION

How does the hypothalamus, when stimulated by a direct or indirect ocular light supply, regulate hypophyseal functions? I shall try to answer

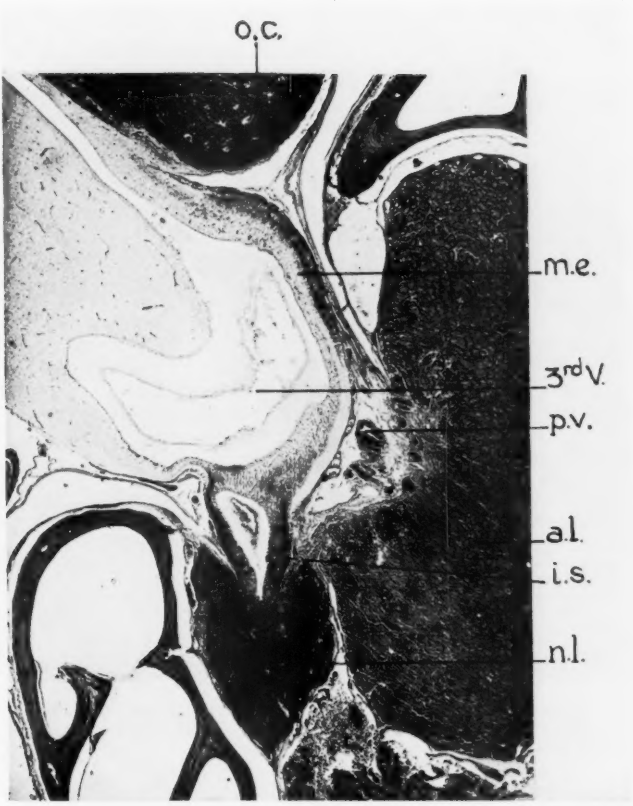


FIG. 7. Median sagittal section through the hypophyseal complex, showing the extension of the FP-positive "special zone" of the median eminence. a.l., anterior lobe; i.s., infundibular stalk; m.e., median eminence; n.l., neural lobe; o.c., optic chiasm; p.v., portal veins; 3rdV., third ventricle. Fuchsin paraldehyde. Magnification: x23.

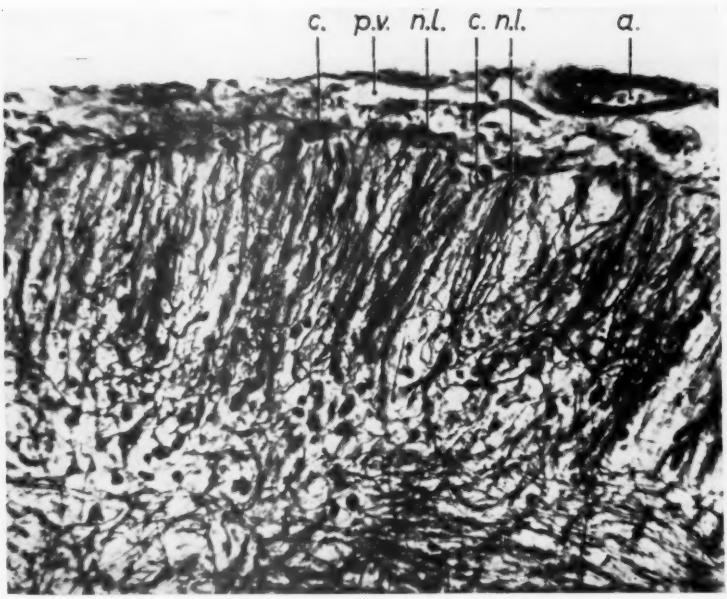


FIG. 8. Superficial layer of the duck's median eminence, at the level of the special zone containing nerve loops. c = capillary; p.v. = portal vein; n.l. = nerve loop; a = artery. (Bodian impregnation technique).

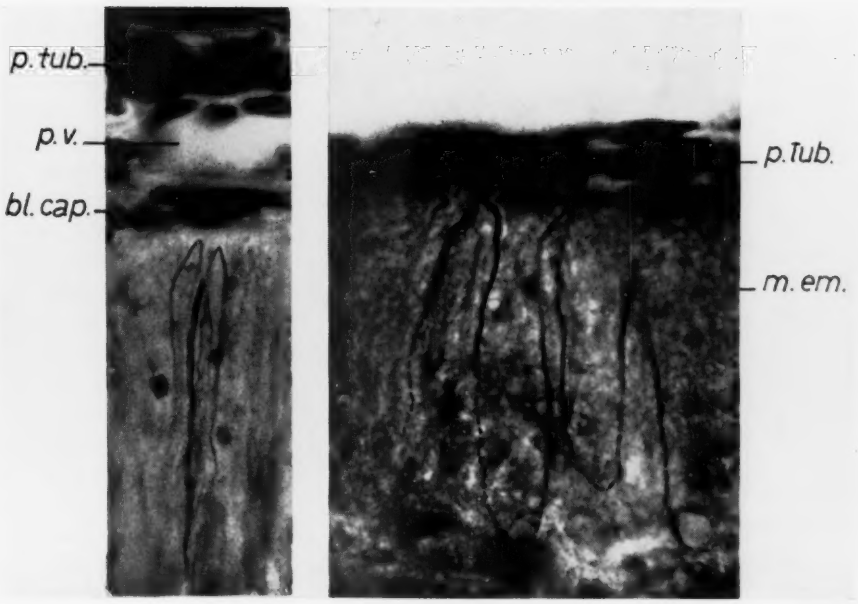


FIG. 9. Nerve loops (n.l.) in the "special zone" of the median eminence (m.e.); p.c.p., primary capillary plexus; p.t., pars tuberalis. Bodian impregnation technique. Magnification: x1040.

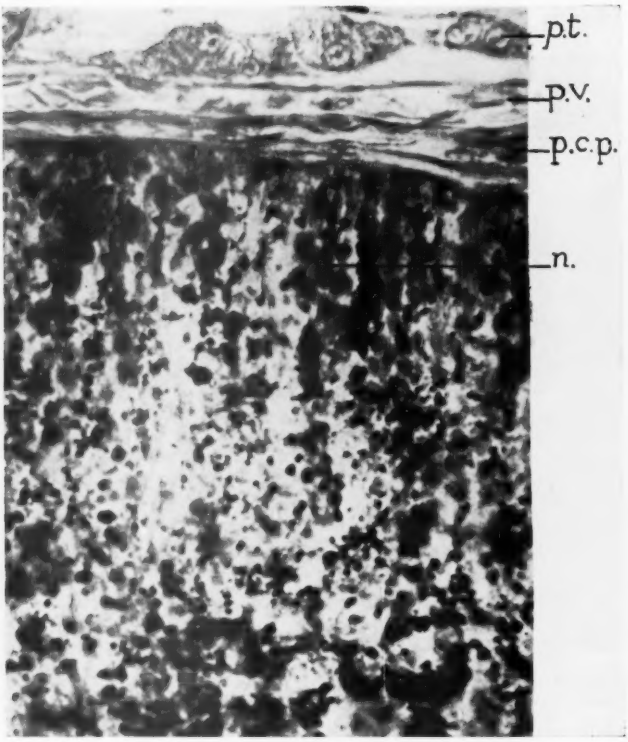


FIG. 10. "Special zone" of the median eminence. n., FP—positive neurosecretory material; p.c.p., primary capillary plexus of the portal system; p.t., pars tuberalis; p.v., portal vein. Fuchsin paraldehyde. Magnification: x400.

this question on the basis of both morphological and physiological studies performed by Dr. Assenmacher and myself.

Anatomical links between the hypothalamus and the pars distalis of the pituitary. Dr. Assenmacher and I have studied the anatomical links between the hypothalamus and the pars distalis in ducks. Using the Bodian impregnation technique, we were not able to establish the existence of a direct nervous link, but the study of the vascularization of this region enabled us to describe a very remarkable link—the *hypophyseal portal system* not previously described in the birds[19, 20] (Fig. 7). All, or nearly all, the blood reaching the sinusoidal capillaries of the pars distalis (secondary capillary plexus) passed first through about twenty-five portal vessels which drained a highly developed capillary network (primary capillary plexus). This network was spread over the surface of the median eminence and received blood from small arteries derived from the internal carotids.

The superficial layer of the median eminence contained a very peculiar disposition of nerves and *neurosecretory fibers* that were in close contact with the primary capillary plexus. In the first place, there were numerous nerve fibers originating in the anterior hypothalamic nuclei and descending through the supraoptico-posthypophyseal tract to end in loops near the blood vessels[20, 23, 24] (Figs. 8 and 9).

Secondly, there were a great number of *granules* at the surface of these loops, *granules* that stained with Gomori's Chrom-hematoxylin-phloxin (CHP) or by Gomori's fuchsin paraldehyde (AF) (Fig. 10). The granules originated in the supraoptic and paraventricular nuclei;[25, 26] no other hypothalamic nucleus contains such abundant CHP- and AF-positive material.

INFERENCES

These structures strongly support the theory of a neurohumoral mechanism in the hypothalamic control of the anterior lobe, which was suggested by Green and Harris in 1947.[29] As a working hypothesis, we may postulate that certain substance(s) elaborated by hpothalamic nuclei are transported by nerve fibres towards the primary capillary net and to glandular cells of the adenohypophysis.

EXPERIMENTS CONCERNING THE HYPOTHALAMIC NEUROHUMORAL CONTROL OF GONADOTROPIC FUNCTION

The peculiar distribution of both vascular and nervous structures in the duck's pituitary, in particular the fact that blood is supplied to the gland through portal vessels located two millimeters ahead of the hypophyseal

stem, makes it possible to perform some crucial experiments in this species. For instance, different types of hypothalamo-hypophyseal transections may be performed to determine the nature and extent of the hypothalamic control over the adenohypophyseal gonadotropic function. The

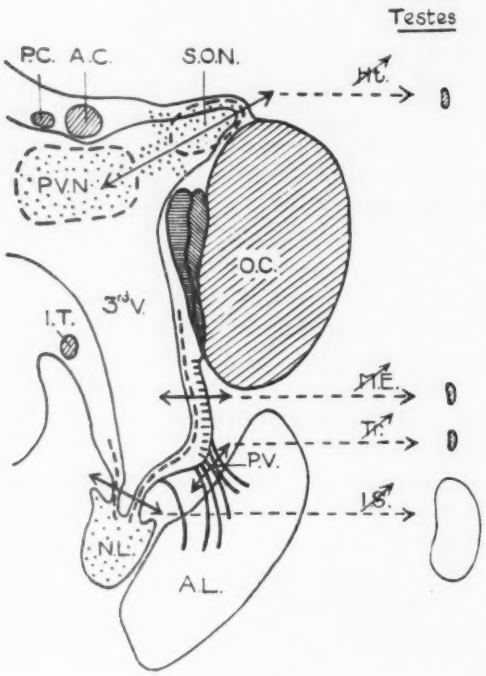


FIG. 11. Schema of the different operations performed on the hypothalamohypophyseal region of the duck, with their effect on the testes. Ht (with arrow), lesion of the anterior hypothalamus; M.E. (with arrow), section of the hypothalamohypophyseal tract in the median eminence; Tr (with arrow), section of the portal veins in the portotuberal tract; I.S. (with arrow), section of the hypothalamohypophyseal tract in the infundibular stalk; A.C., anterior commissure; A.L., anterior lobe; I.T., infundibular tract; N.L., neural lobe; O.C., optic chiasm; P.C., pallial commissure; P.V., portal veins; P.V.N., paraventricular nucleus; S.O.N., supraoptic nucleus; 3rd V., third ventricle.

discrepancies between control values and the effect of such interventions would indicate the involvement of the hypothalamus (Fig. 11). This is not possible in mammals because a section of the portal vessels cannot be performed without severing at the same time the nerve fibers of the hypothalamo-hypophyseal tract.

FIG. 12. Left testes of three operated ducks. From top to bottom: section of the portal vessels; transection of the median eminence; transection of the infundibular stalk. (Magnification: x1).

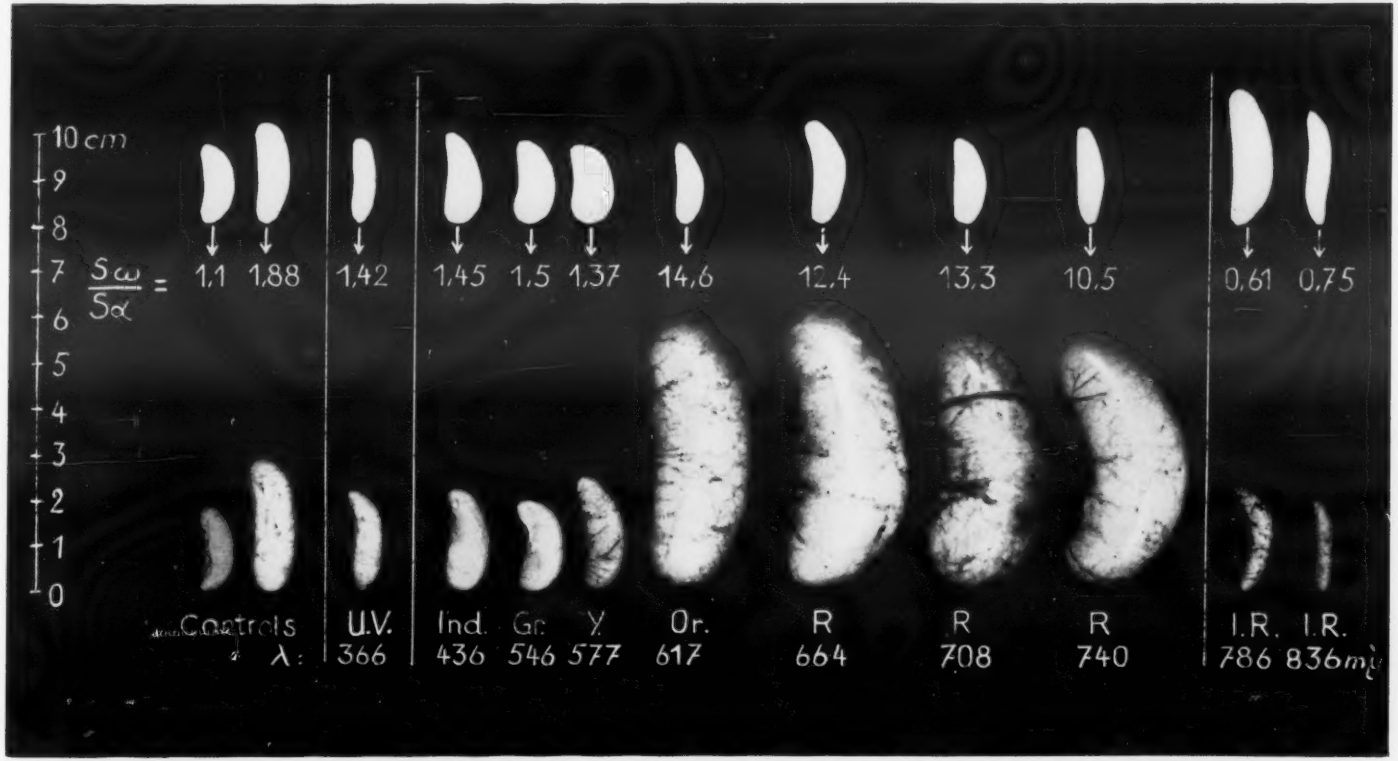


FIG. 13. Effect of illumination of various wavelengths on the gonadal-stimulating mechanisms of the duck. Above: outline of left testes drawn before the experiment. Below: the same, photographed at autopsy. The ratio Sω/Sα is indicative of changes in the testicular outline during the experiment. Only orange and red radiations stimulated the testicular growth (monochromatic wavelengths extending from the ultraviolet to the red region were supplied by a vapor lamp; orange and red radiations were obtained from an incandescent lamp provided with interference filters).

1. *Pituitary Grafts.* Pituitary homografts placed at a distance from the median eminence of male adult ducks hypophysectomized by Dr. Assenmacher were used to obtain one type of total hypothalamo-hypophyseal interruption. Nine ducks survived in very good condition, thanks to the graft, and were sacrificed two and a half months to six months after surgery. No significant pituitary was found at autopsy in the sella turcica and all grafts were well vascularized. The pituitary cells were degranulated and small and although well vascularized and living, they seemed to have undergone a process of "dedifferentiation" as a result of the absence of hypothalamic inducing factors. In all animals the testes were atrophic even when light was given prior to autopsy. This demonstrates that hypophyseal tissue, when deprived of its apical blood supply from the median eminence, is not capable of maintaining a normal gonadotropic function.

2. *Section of the hypophyseal portal vessels.*[22,30-32] In this experiment (26 cases) a piece of sclera taken from the resected eye was introduced between the median eminence and the pituitary, after section of the portal vessels, in order to prevent regeneration of the portal vessels. Thus the adenohypophysis was deprived of blood that had circulated in the "special zone" of the median eminence to reach the gland mainly through the anterior portal veins.

The results of this intervention could be correlated with the vascularization of the hypophysis at autopsy. When all anterior portal veins were severed, or no vascular regeneration occurred between the median eminence and the gland, then the testes were atrophic and the photosexual reflex was abolished (Fig. 11 and 12). Moreover, hemicastration was never followed by compensatory hypertrophy of the remaining gonad. In normal conditions, compensatory hypertrophy results from an increase in hypothalamic stimulation of the pituitary, an effect which follows a lowered blood level of testicular hormones. Thus the absence of testicular hypertrophy in ducks with sectioned portal veins can be interpreted as a blockade of the transmission of hypothalamic influences. When some portal veins were left intact, or when some regeneration of the vessels occurred, the testes did not involute and the photosexual reflex remained normal.

It could be objected that in some animals of this group, the interruption of the portal blood supply had brought about a noticeable atrophy of the cephalic lobe of the pars distalis, where necrotic zones were at times observed. However, this does not seem to account for the results observed for several reasons. i) If we could exclude the aforementioned necrotic zones, the parenchyma of the adenohypophysis appeared uninjured. The cells were small, but the vascularization was good. ii) The amount of living

parenchyma (60 to 85% of the total gland) exceeded the quantity reportedly required to insure full development of both seminiferous and interstitial tissue. In partially gonadectomized ducks, twenty per cent of the remaining pituitary tissue induced complete development of the gonads. iii) The genodotropic function did not seem to be exclusively dependent upon the cephalic lobe of the adenohypophysis. When implanted in immature mice, both the cephalic and the caudal lobes had gonad-stimulating activity.

We assumed, therefore, that the genital atrophy observed after complete severance of the hypophyseal-portal vessels was best accounted for by the absence of any vascular connection between the AF-positive zone of the median eminence and the *pars distalis.*

3. *Section of the hypothalamo-hypophyseal tract.*[86] The influence of this intervention was variable according to the level of the section. For example, when the tract was severed in the *infundibular stalk,* the posterior lobe atrophied completely. However, the median eminence, the portal system, and the anterior lobe remained normal, and the gonadotropic function was not disturbed (eight cases, Figs. 11 & 12). However, when the section was performed in the *median eminence* the whole neurohypophysis, including the posterior lobe and the median eminence, involuted. In these conditions the nerve fibers and the neurosecretory material were no longer seen in the median eminence and the gonads rapidly became completely and permanently atrophic (nineteen cases, Figs. 11 and 12).

4. *Lesions of the anterior hypothalamus.*[84] Large bilateral lesions were placed in the supraoptic and the paraventricular regions by means of a direct current coagulator (nineteen cases). Because the neurosecretory material originates in these nuclei their destruction was always followed by genital atrophy with the same latency as after hypophysectomy, an average of two to three weeks (Fig. 11). The median eminence no longer contained AF-positive neurosecretory material. Moreover, hemicastration did not provoke compensatory testicular hypertrophy in these animals.[85] Once the anterior hypothalamic nuclei were destroyed, hypothalamic impulses no longer reached the pituitary to stimulate the gonadotropic secretion.

5. *Cytological study of the pars distalis in control ducks and ducks subjected to operation.* The cytology of the *pars distalis* was studied with staining methods which have been used for many years by Herlant and his collaborators.

Normal hypophysis. The hypophyseal cytology of birds has been studied by several authors: Schooley and Riddle,[86] Rahn and Painter,[87] Painter,[88] Payne,[89-43] Wingstrand,[42] Legait and Legait,[44,45] and Mikami.[47] Recently, with

Herlant *et al.*[48] we were able to distinguish six cellular types in the duck's pituitary; five of them were homologous to those described by Herlant in a dozen mammals. According to Herlant's terminology, the acidophilic cells are referred to as alpha and epsilon, and the glycoprotein-containing cells as beta, gamma, and delta. Three types of cells appeared to be gonadotropic; one of them, located exclusively in the caudal lobe of the pituitary, turned carmine when stained with azan, purple with Herlant's dye, light blue with the methasol blue-PAS method, and is PAS-positive. These cells became active from December through April which is during the time of testicular growth. They involuted during testicular regression. The close parallelism between the testicular cycle, particularly the interstitial cells, and the secretory cycle of this hypophyseal type, enables us to consider it as LH-producing.

The beta cells, located in the cephalic lobe, were also PAS-positive and purple when stained with Herlant's dye; but they turned violet-blue with the methanol blue-PAS and were not carminophilic. Their activation and regression took place at a later period than it did for the gamma cells (February through July); they are believed to secrete FSH.

Epsilon cells, also located in the cephalic lobe, were PAS-negative, erythrosinophilic when stained according to Herlant's technique, and light blue with the methanol blue-PAS. They underwent cyclical changes very similar to those described for the gamma cells, and they seem to correspond to LTH secretion.

These cytological data enabled us to interpret the changes brought forward by various surgical interventions on the hypothalamo-hypophyseal complex of the duck.

Hypophysis of ducks with section of the portal veins, a transection of the median eminence, or hypothalamic lesions. Since Bouin fixative and a decalcifying solution of trichloracetic acid had always been used for the hypothalamo-hypophyseal regions of ducks subjected to surgery, Herlant's staining method with alizarin blue had to be slightly modified. In unpublished data Tixier and Assenmacher observed that pituitary cells underwent, to a varying extent, the same type of changes after section of the anterior portal vessels, transection of the hypothalamo-hypophyseal tract within the "special zone" of the median eminence, or lesions of the magnocellular nuclei. These changes involved mainly a volume reduction and a differentiation of the cellular bodies. However, the several cellular types showed a different sensitivity to these interventions. They may be classified as follows, in a decreasing order of sensitivity: prolactin cells (ϵ), FSH

cells (β), TSH cells (δ), LH-cells (γ) and fiinally α-cells. Only the H-cells (MSH ?) seemed unaffected by the surgery.

COMMENT

We may conclude from these experiments that in all ducks with genital involution and loss of photo-sexual reflex, the natural connection was interrupted between the "special zone" of the median eminence (with its nerve loops and its neurosecretory material) and the hypophyseal portal system. This interruption may be caused either by a vascular lesion such as section of the hypophyseal portal vessels or hypophyseal graft after hypophysectomy, or it may be caused by a nervous lesion such as complete section of the hypothalamo-hypophyseal tract at the level of the median eminence or anterior hypothalamic lesion.

The following observations support the theory of a neurosecretory humoral path for the photo-sexual reflex. i) In the duck, the photo-sexual reflex requires the absolute integrity of the connections between the hypothalamus and the adenohypophysis. ii) The hypothalamic control of the gonadotropic function seems to involve a neurohumoral mediator at the level of the "special zone" of the median eminence. This mediator, probably linked to the AF-positive, neurosecretory material, migrates from the hypothalamic nuclei into the hypophyseal portal plexus to activate the gonadotropic function.

Although genital functions were completely abolished by experimental hypothalamo-hypophyseal interruption, the thyroid and the adrenal cortex were scarcely affected by the intervention.[29] This fact suggests, as did the aforementioned observations concerning the differential response of the various adenohypophyseal cells to 'interruption' experiments, that there may be several hypothalamic neurohumoral mediators.

Oksche, Laws, and Farner in 1958[40] drew similar conclusions from experiments on *Zonotrichia leucophrys gambelii.* AF-stained neurosecretory material was found in large amounts in the median eminence of birds submitted to artificial "short days" (eight hours of light daily). No testicular growth occurred in these animals. However, following the transfer of the animals to an environment with a photoperiodicity of 20 hours a day rapid testicular growth occurred. In the light of these data, the authors suggested that the stainable neurosecretory material in the median eminence contained an agent which causes the anterior pituitary to release gonadotropins. Similarly, Kobayashi, Wolfson, Wise, and Haubrich[60] reported variations in the acid phosphatase activity of the median eminenece and the supraoptic nucleus in birds given artificial light for sixteen hours a day; the acid

phosphatase content paralleled a rapid growth of the testes and returned to the initial value when the testicular size decreased.

SENSITIVITY OF THE PHOTORECEPTORS IN THE PHOTO-SEXUAL REFLEX

Now that we have reviewed the rôle of three organs involved in the photo-sexual reflex—the eye, the hypothalamus, and the pituitary—let us consider in greater detail the respective sensitivity of the two types of photoreceptors referred to earlier.

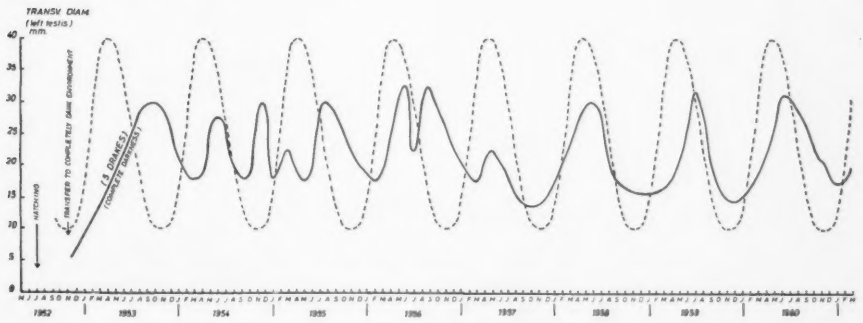

TRANSV. DIAM. (left testis) mm.
40
35
30
25
20
15
10
5
0
HATCHING
TWO YEARS TO COMPLETED DARK ENVIRONMENT
(4 DRAKES)
(COMPLETE DARKNESS)
M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M
1952
1953
1954
1955
1956
1957
1958
1959
1960

FIG. 14. Testicular changes in five ducks kept for 8½ years in a completely dark environment (the experiment was started when the animals were 4 to 4½ months old). The cycles are irregular and shorter than the normal testicular cycle during the four first years; towards the end of the experiment, they surprisingly tend to parallel the yearly rhythm observed in the controls, and show a peak between May and July (unpublished curve from Benoit, Assenmacher and Brard).

Light-sensitivity of the retina vs. visual sensitivity. The light-sensitivity of the retina in the photo-sexual reflex seemed to differ in two respects from its visual sensitivity. For example, it may be inferred from experiments in which drakes were exposed to radiations, not only of different wavelengths, but of equal energies, only orange and red rays were effective in stimulating testicular growth.[51,52, also 53–56] In particular, yellow radiations, which were most active on vision were totally ineffective for gonad-stimulation (Fig. 13).[57]

In further contrast, the receptors of the photo-sexual reflex seemed to be much less sensitive than the visual receptors. They were stimulated in the duck, as in many other birds, by threshold energies in the range of 1 to 5 lux, whereas the threshold for visual stimulation, in man for instance, equals about one millionth of this value.

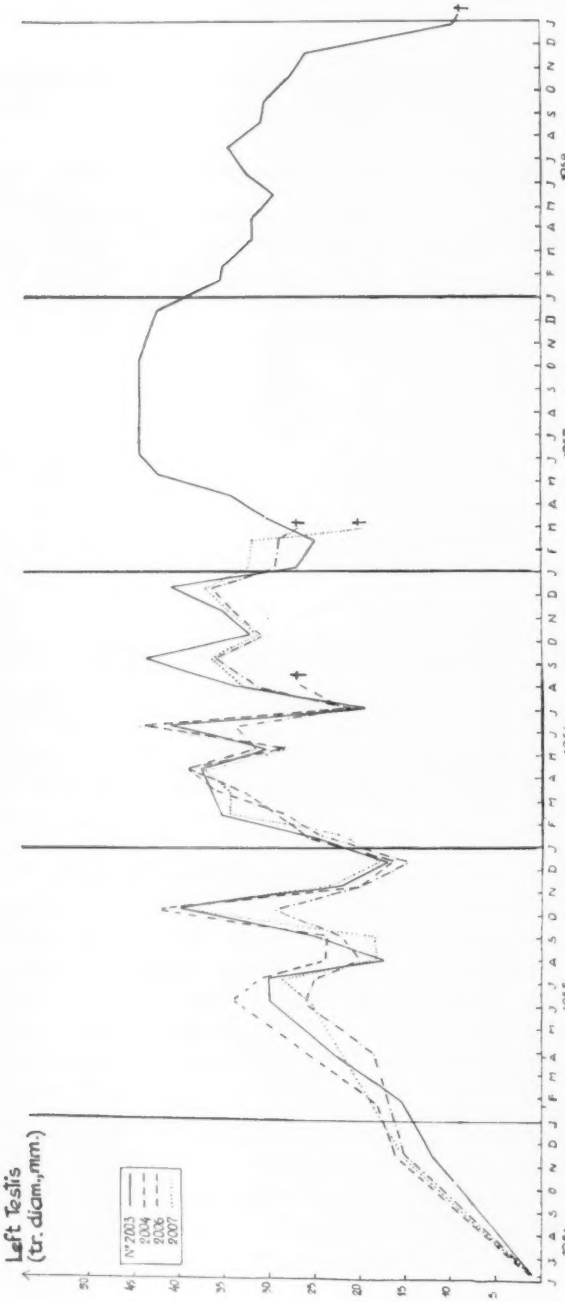


FIG. 15. Testicular development in four Pekin ducks kept under constant light from the age of 18 days on .The cycles are irregular and shorter than the normal cycle. Note the synchronism of the cycle within the group and the high "plateau"-shaped curve of the last animal after the death of the other ducks in the group.

These differences suggest a fundamental difference in the two functions; the retinal processes involved in gonad-stimulation have in fact already been considered as a *neurovegetative function* by some biologists.

What is the anatomical substrate of this neurovegetative function? This very important problem has not yet been given much attention. Becher[58,59] nevertheless postulated that in mammals, special vegetative rentinal neurons, smaller than the ganglion cells of the eighth retinal layer but spread out

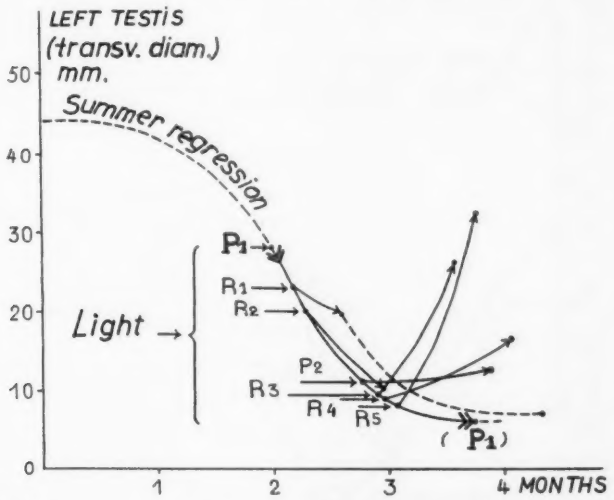


FIG. 16. Transverse diameter of the left testis in ducks submitted to a strong supply of light during the refractory period (solid lines). In R1, testicular regression resumed after interruption of treatment on the twelfth day (dotted line); in P1, the treatment was not only ineffective, but blocked subsequent development of the testes (arrows).

among them, could be the specific receptors of the photo-sexual reflex; their axons would be directly connected with the hypothalamus.

Sensitivity of the hypothalamus to direct visible radiations. The hypothalamus seemed to be sensitive to the whole spectrum, or at least to indigo, blue, yellow, and red rays, in contrast to the retina which, as we have seen, was stimulated only by orange and red radiations. The hypothalamic response to all these aforementioned colored radiations was recorded with the quartz rod stimulation method.[7,51,52]

Apart from the striking difference between the "neurovegetative" retina and the hypothalamic photoreceptor, it was demonstrated that the sensitivity of the hypothalamus to light is even greater than that of the peripheral receptor.[60]

HYPOTHALAMIC CONTROL OF THE PITUITARY IN DIFFERENT PHYSIOLOGICAL CONDITIONS

The experiments involving complete severance of all connections between the median eminence and the adenohypophysis demonstrated that the gonadotropic secretion is entirely under hypothalamic control.

But how does the hypothalamo-hypophyseal complex behave when birds are completely deprived of light?

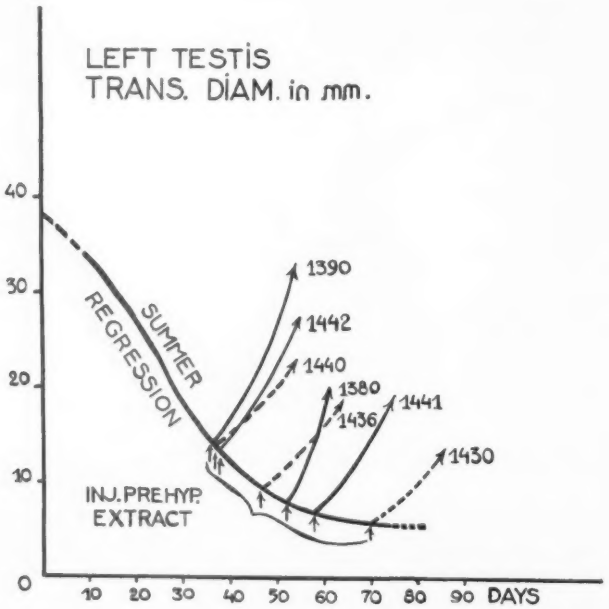


FIG. 17. Injection of bovine adenohypophyseal extracts to seven male ducks during the testicular regression period. The curves account for the increase in transverse diameter of the left testis consecutive to treatment.

Thirteen ducks were transferred to a *completely dark environment* at ages varying from 18 to 135 days. Five of them were kept there for over eight years. The total absence of light did not prevent them from reaching sexual maturity with normal cycles although the onset of puberty occurred later than in control animals, and the cycles were shorter and irregular (from 3 to 12 months). During the first five years these cycles did not parallel the seasons and were less accentuated than in normal conditions (minimal and maximal average transverse diameters of the left testis: 16 and 32 mm. versus 8 and 45 mm.)[41, 62] (Fig. 14). During the last four

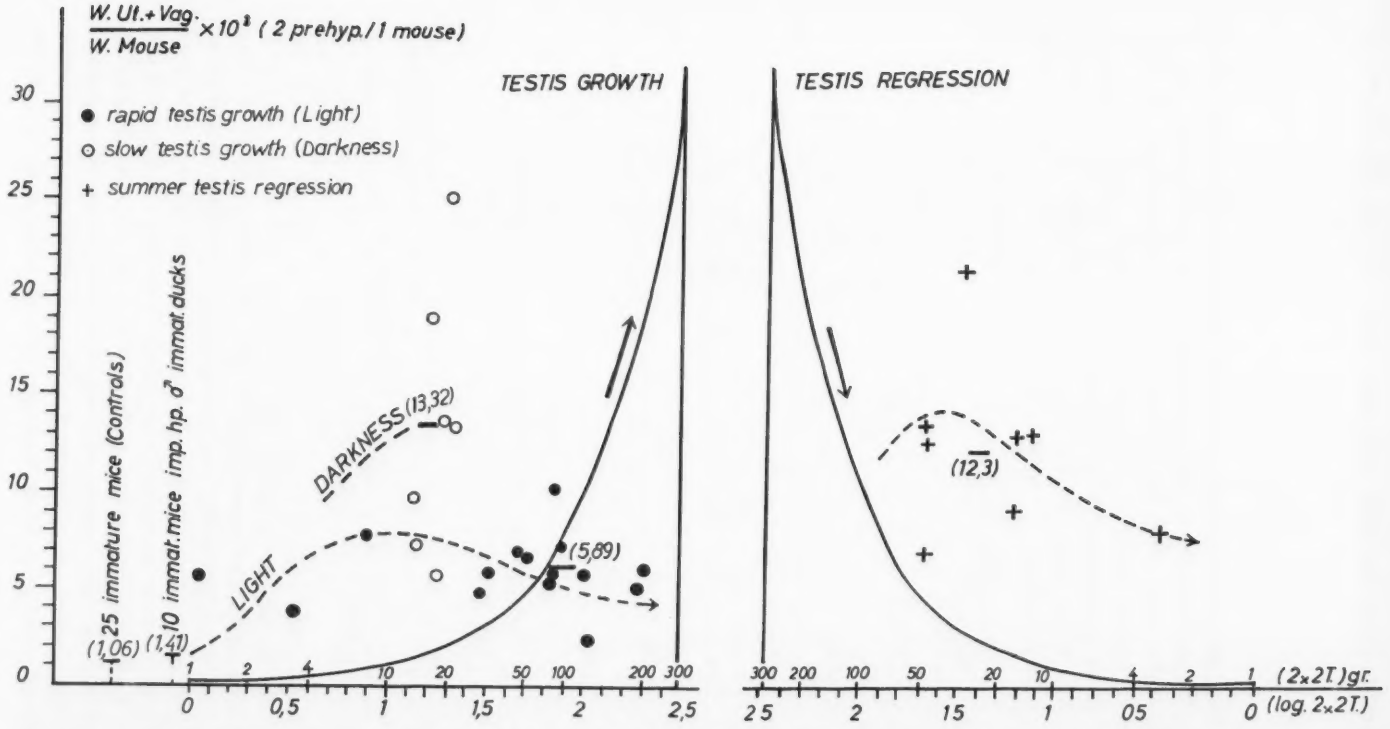


FIG. 18. Seasonal variations in the gonadotropic potency of pituitaries implanted to immature female mice. Note that a light gonadotropic potency parallels a slow testicular growth in animals kept in the dark, whereas a low gonadotropic content corresponds to a fast testicular growth in animals kept under permanent light. The gonadotropic release appears thus to be inhibited by darkness and enhanced by light. The period of testicular regression (refractory phase) is characterized by a storage of gonadotropins, which are no longer released from the pituitary.

years the cycles tended to follow the normal periodicity. We are not able to explain this phenomenon. A certain synchronism was noted in the cycles of these animals, a fact which might imply the intervention of psychological factors. It appeared that light, if not absolutely essential to sexual activity, accounted for the seasonal periodicity observed in birds' gonads. There may be internal factors related to the properties and functions of the hypothalamus, the hypophysis, the gonads, the thyroid, and other organs, which

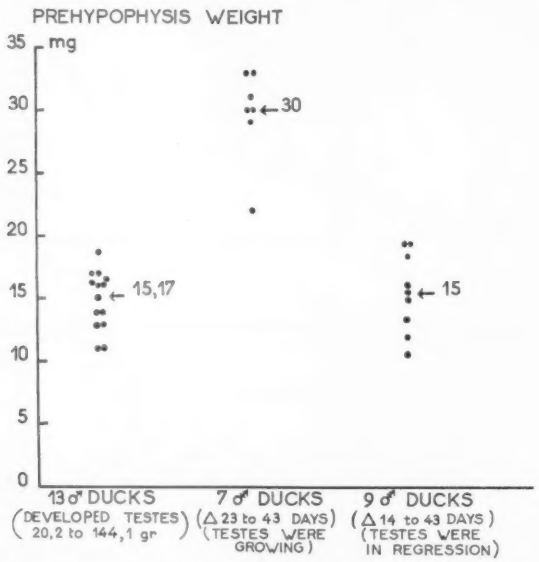


FIG. 19. Weight of adult male adenohypophyses from: (i) 13 normal subjects with growing testes; (ii) 7 subjects gonadectomized at the time of testicular development; (iii) 9 subjects gonadectomized at the time of testicular regression.
Note that gonadectomy brings about a pituitary hypertrophy only when testes are removed during their normal growing period.

may involve psychological factors and interact to impose a given but variable rhythmicity to the genital activity.

When exposed to *continuous artificial light* from the age of eighteen days on, ducks have presented for three years, irregular and short sexual cycles similar to those observed in animals deprived of light. These cycles were even shorter in some instances[63,64] (Fig. 15). Uninterrupted light was not capable of maintaining the gonad-stimulating activity of the pituitary at its maximum. On the contrary, after a certain point, the gonads regressed even if the illumination was continued. This probably happens in natural conditions evidenced by the regressing gonads observed at the end of the

period of intense sexual activity. Supplementary artificial light does not prevent the spontaneous regression; the animals are in a "refractory period" in which light is no longer effective (Fig. 16[85] and authors who deal with various other species of birds[58, 65–67]).

Which one of the three organs: the hypothalamus, the pituitary, or the gonad, is responsible for this effect? The *testis* is not responsible for the

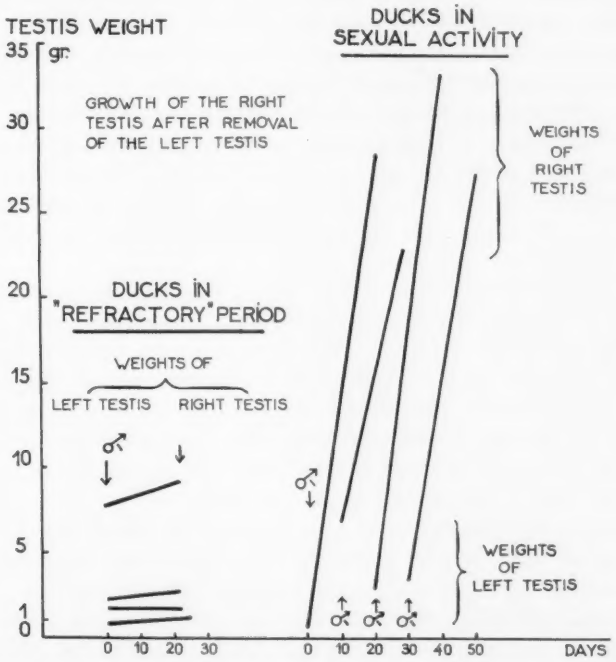


FIG. 20. Compensatory hypertrophy of the right testis after hemicastration in ducks operated during or after the refractory period.

refractory period, since it responds to an injection of pituitary extracts[68–70] (Fig. 17). Its sensitivity to gonadotropic hormones might nevertheless be diminished during the regression period—it has indeed been noted in the female Sparrow that the gonads respond less[71] or more slowly[72] to serial gonadotropins during the refractory period.

The hypothalamus and the adenohypophysis might account for the refractory period and the following experiments can help us settle the question.

As the testes regress to a complete resting state during the refractory period, one can postulate that gonadotropins are, at that time, either missing

or present at subthreshold levels in the blood. Furthermore, pituitary glands taken from ducks during the refractory period, then implanted in immature female mice, showed a higher gonadotropic potency than when taken during the period of sexual activity (Fig. 18). Siminarly, the hypophysis of the pheasant was particularly rich in gonadotropins between August and January.[78] These experiments demonstrated that during the refractory period the release of gonadotropic hormones from the pituitary is considerably diminished or even stopped.

Bilateral castration, which causes a notable increase in volume of the adenohypophysis (100%) when the testes are active, did not result in any increase of the pituitary's volume when it was performed during the refractory period (Fig. 19). At this time too, hemicastration was not followed by hyperactivity of the pituitary and by compensatory hypertrophy of the remnant testis (Fig. 20). Section of the portal veins or destruction of the anterior hypothalamus, followed by unilateral castration, did not lead to hypertrophy of the other testis.[22]

All these results seemed to indicate that the hypothalamus and not the pituitary was responsible for the regression of the sexual activity during the refractory period. However, the definitive answer will not be known until the isolation of the gonadotropic releasing factor is accomplished and it is injected into birds during the refractory period. Then we may know if the pituitary is still able to secrete the gonadotropins or not. A positive response will establish without doubt that the refractory period is caused by a lessening of activity of the hypothalamus.

Implanting the pituitary yielded additional information concerning the release of the gonadotropins. When immature female mice were implanted with pituitaries taken from immature ducks maintained in a dark environment, ducks with slowly growing testes, the response of the genital tract of the mouse was strong indicating that the gonadotropic content of the pituitary was high. When implantation was made of pituitaries coming from illuminated immature ducks, the response of the mouse was minimal; gonadotropic content was low (Fig. 18).

Light appears, therefore, to enhance both secretion and excretion of gonadotropins, whereas the absence of light inhibits or stops their excretion. The hypothalamus itself may respond to variations in the amount of light and thus regulate the adenohypophyseal activity.

CONCLUSIONS

The results I have presented to you are demonstrative of the important role of the hypothalamo-hypophyseal complex in the photo-sexual reflex.

Pituitary gonadotropic activity seems to be entirely regulated by the hypothalamus in the duck, as probably in all birds, through a neurohumoral mechanism involving one or several hypothalamic mediators related to the hypothalamic neurosecretion.

From a more general point of view, one should remember that the hypothalamus controls not only sexual activity, but, to a varying extent, the activity of the other endocrine organs and some of the most important autonomic functions. Some effects of light on blood pressure, diuresis, and glycemia, for instance, are well known. The importance of the hypothalamus for the autonomic system on one hand, and its close connections to the eye on the other, suggest that the study of the photo-sexual reflex should be extended to other photo-endocrine and to autonomic processes. Biologists would thus confront a more coherent and extensive field, and such an approach to the action of light upon the organism as a whole would correspond more closely to objective physiological conditions.

REFERENCES

1. Benoit, J.: *C. R. Acad. Sci. (Paris)*, 1934, *199*, 1671.
2. Benoit, J.: *C. R. Soc. Biol. (Paris)*, 1935, *118*, 664.
3. Benoit, J.: *C. R. Acad. Sci. (Paris)*, 1935, *201*, 359.
4. Benoit, J.: *Bull. biol. Fr. Belg.*, 1936, *70*, 487-533.
5. Benoit, J.: *C. R. Soc. Biol. (Paris)*, 1936, *120*, 1236.
6. Benoit, J.: *C. R. Soc. Biol. (Paris)*, 1935, *118*, 669.
7. Benoit, J.: *C. R. Soc. Biol. (Paris)*, 1938, *127*, 909.
8. Benoit, J.: *C. R. Soc. Biol. (Paris)*, 1938, *129*, 231.
9. Benoit, J.: *C. R. Soc. Biol. (Paris)*, 1935, *120*, 133.
10. Benoit, J.: *C. R. Soc. Biol. (Paris)*, 1935, *120*, 136.
11. Benoit, J.: *Rev. Physiothér.*, 1936, *2*, 86-94.
12. Benoit, J. and Kehl, R.: *C. R. Soc. Biol. (Paris)*, 1939, *131*, 89.
13. Frisch, K. von: *Pflugers Arch. ges. Physiol.*, 1911, *138*, 319-387.
14. Scharrer, E.: *Z. vergl. Physiol.*, 1928, *7*, 1-38.
15. Rodewald, W.: *Z. vergl. Physiol.*, 1928, *7*, 1-38.
16. Benoit, J. and Assenmacher, I.: *J. Physiol. (Paris)*, 1953, *45*, 34-37.
17. Benoit, J., Assenmacher, I., and Walter, F. X.: *C. R. Soc. Biol. (Paris)*, 1953, *147*, 186.
18. Benoit, J., Assenmacher, I., and Manuel, S.: *C. R. Acad. Sci. (Paris)*, 1953, *235*, 1695.
19. Benoit, J., Assenmacher, I., and Manuel, S.: *C. R. Soc. Biol. (Paris)*, 1953, *147*, 40.
20. Benoit, J., Tauc, L., and Assenmacher, I.: *C. R. Acad. Sci. (Paris)*, 1954, *239*, 451-453.
21. Benoit, J., Tauc, L., and Assenmacher, I.: *C. R. Acad. Sci. (Paris)*, 1954, *239*, 508-510.
22. Benoit, J. and Assenmacher, I.: *Bull. Histol. appl.*, 1950, *27*, 182.
23. Benoit, J. and Assenmacher, I.: *Arch. Anat. micr. Morphol. exp.*, 1951, *40*, 27-45.
24. Assenmacher, I.: *Arch. Anat. micr. Morphol. exp.*, 1952, *41*, 69 et 107.
25. Assenmacher, I. and Benoit, J.: *In: Pathophysiologia diencephalica.* Hrsg. von S. B. Curri u.L. Martini. Wien, Springer-Verlag, 1958, in -8°, pp. 401-427.
26. Benoit, J. and Assenmacher, I.: *C. R. Assoc. Anat.*, 1951, *65*, 154-160.
27. Benoit, J. and Assenmacher, I.: *C. R. Soc. Biol. (Paris)*, 1951, *145*, 1395.
28. Assenmacher, I.: *Arch. Anat. micr. Morphol. exp.*, 1958, *47*, 448-572.
29. Assenmacher, I. and Benoit, J.: *C. R. Acad. Sci. (Paris)*, 1953, *236*, 133.

30. Benoit, J. and Assenmacher, I.: Rapport IIe Reun. Endocr. de langue française. Paris, Masson, 1953, p. 33.
31. Benoit, J. and Assenmacher, I.: *Publ. Staz. zool. Napoli.*, 1954, *24*, 27-31.
32. Green, J. D. and Harris, G. W.: *J. endocr.*, 1947, *5*, 136.
33. Assenmacher, I.: *Arch. Anat. Histol. Embryol.*, 1953, *35*, 181-202.
34. Assenmacher, I. and Benoit, J.: *C. R. Acad. Sci. (Paris)*, 1953, *236*, 2002.
35. Assenmacher, I. and Benoit, J.: *C. R. Acad. Sci. (Paris)*, 1956, *242*, 2986.
36. Benoit, J. and Assenmacher, I.: *C. R. Acad. Sci. (Paris)*, 1952, *235*, 1547.
37. Assenmacher, I.: *C. R. Acad. Sci. (Paris)*, 1957, *245*, 210-213.
38. Benoit, J., Assenmacher, I., and Walter, F. X.: *C. R. Soc. Biol. (Paris)*, 1950, *144*, 573.
39. Schooley, J. P. and Riddle, O.: *Amer. J. Anat.*, 1938, *62*, 313-349.
40. Rahn, H. V. and Painter, B. T.: *Anat. Rec.*, 1941, *79*, 297-311.
41. Painter, F.: *Anat. Rec.*, 1942, *84*, 387-405.
42. Payne, F.: *Biol. Bull.*, 1942, *82*, 79-111.
43. Payne, F.: *Anat. Rec.*, 1943, *86*, 1-13.
44. Payne, F.: *Anat. Rec.*, 1946, *96*, 77-91.
45. Payne, F.: *Anat. Rec.*, 1955, *122*, 49-56.
46. Wingstrand K. G.: *The structure and development of the avian pituitary.* Lund, C. W. K. Gleerup, 1951, p. 316.
47. Legait, H. and Legait, E.: *41ème Reunion Assoc. Anat., Genes*, 1954, *84*, 188-199.
48. Legait, H. and Legait, E.: *C. R. Soc. Biol. (Paris)*, 1955, *149*, 2207-2209.
49. Legait, H. and Legait, E.: *C. R. Assoc. Anat.*, 1956, *91*, 902-908.
50. Mikami, E.: *J. Fac. Agric. Iwate Univ.*, 1958, *3*, 473-545.
51. Herlant, M., Benoit, J., Tixier-Vidal, A., and Assenmacher, I.: *C. R. Acad. Sci. (Paris)*, 1960, *250*, 2936-2938.
52. Oksche, A., Laws, D., and Farner, D. S.: *Anat. Rec.*, 1958, *30*, 433.
53. Kobayashi, H., Wolfson, A., Wise, M. A., and Haubrich, D. R.: *Anat. Rec.*, 1960, *137*, 372.
54. Benoit, J., Walter, F. X., and Assenmacher, I.: *C. R. Soc. Biol. (Paris)*, 1950, *144*, 1026.
55. Benoit, J., Walter, F. X., and Assenmacher, I.: *J. Physiol. (Paris)*, 1950, *42*, 537-541.
56. Bissonnette, T. H.: *Physiol. Zool.*, 1931, *4*, 542-574.
57. Kirschbaum, A.: *Anat. Rec.*, 1933, *57*, suppl., 62.
58. Ringoen, A. R.: *Amer. J. Anat.*, 1942, *71*.
59. Burger, J. W.: *J. exp. Zool.*, 1943, *94* ,161-168.
60. Benoit, J., Assenmacher, I., and Walter, F. X.: *C. R. Soc. Biol. (Paris)*, 1952, *146*, 1027.
61. Becher, H.: Verhandl. Anat. Gesellsch. 51. Versamml., Mainz, 1953, pp. 166-183.
62. Becher, H.: *Acta neuroveg. (Wien)*, 1954, *8*, 421-436.
63. Benoit, J. and Ott, L.: *Yale J. Biol. Med.*, 1944, *17*, 27-46.
64. Benoit, J. and Assenmacher, I.: *C. R. Acad. Sci. (Paris)*, 1954, *239*, 105-107.
65. Benoit, J., Assenmacher, I., and Brard, E.: *C. R. Acad. Sci. (Paris)*, 1955, *241*, 251-253.
66. Benoit, J., Assenmacher, I., and Brard, E.: *J. Physiol. (Paris)*, 1956, *48*, 388-391.
67. Benoit, J., Assenmacher, I., and Brard, E.: *C. R. Acad. Sci. (Paris)*, 1956, *242*, 3113-3115.
68. Benoit, J., Assenmacher, I., and Brard, E.: *Arch. Anat. micr. Morphol. exp.*, 1959, *48* bis, 5-12.
69. Rowan, W.: *Proc. Boston. Soc. nat. Hist.*, 1926, *38*, 147-189.
70. Burger, J. W.: *Bull. Mount. Desert Island biol. Lab.*, 1941,
71. Miller, A. H.: *J. exp. Zool.*, 1948, *109*, 1-11.
72. Clark, L. B., Leonard, S. L., and Bump, G.: *Science*, 1937, *85*, 2005, 339-340.
73. Schildmacher, H.: *Biol. Zbl.*, 1939, *59*, 653-657.
74. Benoit, J., Manuel, S., Walter, F. X., and Assenmacher, I.: *C. R. Soc. Biol. (Paris)*, 1950, *134*, 1400.
75. Riley, G. M. and Witschi, E.: *Endocrinology*, 1938, *23*, 618-624.
76. Vaugien, L.: *Bull. Biol. fr. Belg.*, 1955, *89*, 1-15.
77. Greeley, F. and Meyer, R. K.: *Auk*, 1953, *70*, 350.

ROBIN F. BADGLEY*
MARILYN A. FURNAL**

Department of Social and Preventive Medicine, University of Saskatchewan College of Medicine and Segura Social del Empleado, Lima, Peru

APPOINTMENT BREAKING IN A PEDIATRIC CLINIC†

To maintain organizational efficiency, many clinics schedule their patients by using an appointment system. Several writers have noted the advantages and disadvantages of giving appointments.[1-4] However, few studies have considered a dysfunctional aspect of such systems, such as broken appointments. Hansen,[5] in her study of patients attending a child health conference, found that broken appointments increased as the immunization series was completed as well as with age, unfavorable weather, the number of physicians seen, and patients without telephones. Olencki and Reader[6] have related broken appointments to the age, sex, and nationality backgrounds of patients.

This study which is part of a larger survey,[7,8] analyzes 774 appointments given by the Pediatric Out-Patient Department of the Grace-New Haven Hospital from December 1957 to March 1958. The goals and organization of the clinic have been described elsewhere.[9-11] This survey focuses on two questions: "Who breaks appointments?" and "Why do patients break appointments?"

METHOD

Techniques of data collection were pretested on 206 case histories and on 13 individuals who broke appointments. During the survey data were collected from all "first visit" patients (648) who attended the outpatient department and from all individuals (126) from Greater New Haven who broke appointments. The homes of all patients who broke appointments were visited and an attempt was made to find out why the appointments had not been kept.

By definition, a patient broke an appointment when he neither kept it nor notified the clinic that he couldn't keep it. Cancelled appointments are omitted from this analysis (with the exception of comparing these with weather conditions). Also, patients who broke appointments for two days following two heavy snowstorms (circa 12 inches of snow) are not considered here.

---

* Assistant Professor of Social and Preventive Medicine.
** Assesora de Entermeria.
† This study was supported by a post-doctoral fellowship awarded by the Russell Sage Foundation.

Received for publication 31 May 1961.

During the four-month period under study, 126 appointments given by the Department to patients living in Greater New Haven were broken. Although an attempt was made to interview all appointment breakers, two-fifths of the patients could not be contacted. Incomplete visits were due to no response after two visits (26), the patient had moved (9) or an incorrect address had been given (11), no English was spoken in the home (2), and one refusal by a patient to be interviewed.

The City of New Haven has been studied extensively by sociologists[12–14] and the data obtained here are compared to this background information. Hollingshead's *Two Factor Index of Social Position*[15] is used to classify individuals by social class. In this scale which weighs a person's education and occupation, Class I is the highest socio-economic position and Class V is the lowest.

OBSERVATIONS

The results of the study are grouped under the following headings: (1) weather, (2) class and race, (3) age, (4) disease grouping, (5) length of disease, (6) source of referral, and (7) the patient's evaluation of the clinic.

1. *Weather*

Since the study took place during the winter when poor climatic conditions might effect clinic attendance, data on weather conditions were collected. For each day that appointments were given, the weather was coded into one of three categories: (i) poor—rain, sleet or snow; (ii) fair—below freezing to 40° F; and (iii) excellent—above 40° F. Appointments were divided into (a) kept appointments, (b) cancelled appointments and (c) broken appointments.

As might be expected, there is a significant relationship between weather conditions and the percentage of appointments kept if all types of weather are included in the analysis. In poor weather, 39 per cent of the appointments were cancelled or broken. However, if extremely unfavorable weather conditions are excluded (e.g. heavy snow storms), then the percentage of appointments kept was not related to variations in the weather. Under these circumstances, regardless of the weather, 79 per cent of the appointments were kept, 11 per cent were cancelled and 10 per cent were broken. Unfavorable weather and attendance at the clinic were not related to a patient's place of residence in New Haven, class position, or race.

2. *Class and Race*

In this study the number of broken appointments vary significantly with the class position and racial background of individuals. Negroes constituted 5 per cent of New Haven's population, 35 per cent of the Department's

patients, and 49 per cent of those who broke appointments. The class distribution of the Negroes in the three groups was comparable.

On the basis of their distribution in New Haven's population (67.5%), lower class white patients were over-represented at the clinic (88.4%). In contrast to the Negro patients at the Department, the class position of white patients is significantly related to breaking appointments. The findings presented in Table 1 show that lower class white patients break more appointments than upper class white patients. Also,

TABLE 1. BROKEN APPOINTMENTS OF WHITE PATIENTS BY CLASS

	Broken appointments	
Social class	*Expected* %	*Actual* %
I-III	11.6	2.5
IV	42.4	33.3
V	46.0	64.2
Total	100.0	100.0

Chi-square = 9.82, $p < 0.01$.

upper class white patients break fewer appointments than would be expected by their attendance at the Department. The reverse pattern characterizes lower class white patients.

3. *Age*

The variable of age was selected for analysis in connection with broken appointments since it was believed that parents with young children would not be as familiar with the organization of health services as parents with older children. It was expected that the former would break more appointments than the latter group.

The age distribution of patients and appointment breakers in this study was not significantly related to class position or racial background. However, the age distribution, as anticipated, was significantly lower for appointment breakers than it was for patients at the Department. These findings are summarized in Table 2. Over twice as many parents of children under age one break appointments as would be expected by the age distribution of patients at the clinic.

4. *Disease Grouping*

The diseases of patients attending the clinic were classified by Dr. Silvio J. Onesti, Jr. according to the *Standard Nomenclature of Diseases and Operations*.[10] The complaints of patients breaking appointments were also categorized according to the above source. The classification of these diseases and complaints is not completely comparable since the former are diagnoses based upon clinical observations of physicians, whereas the latter are symptoms reported to a public health nurse by appointment breakers.

TABLE 2. BROKEN APPOINTMENTS BY AGE

	Broken appointments	
Age by years	*Expected* %	*Actual* %
Under 1 year	12.1	31.2
2-5 years	34.9	27.3
6-9 years	22.7	18.2
10 +	30.3	23.3
Total	100.0	100.0

Chi-square = 20.57, $p < 0.01$.

Bearing this discrepancy in mind, it is remarkable that the percentage distribution of diseases was virtually comparable for the two groups. The disease groupings similar for patients and appointment breakers were: body as a whole (6.9%), dermatological (13.9%), respiratory (16.2%), digestive (14.8%), nervous system (7.1%), and others (21.1%).

In only one category was marked variation noted. Psychobiologic disorders were reported for 24.5 per cent of the clinic patients in contrast to 12.9 per cent of the appointment breakers.

Additional analysis of the data revealed no other significant relationships between types of diseases and social characteristics of individuals who kept and did not keep appointments.

5. *Length of Disease*

The length of time that a patient had been suffering from a disease or reported complaint varied with class position and racial background. For example, 54.6 per cent of Class I-III white patients at the clinic had disorders of at least a year's duration. In contrast, 40.7 per cent of white Class IV patients and 32.4 per cent of white Class V patients at the clinic

had diseases for a comparable period. This trend by class membership also characterized Negro patients at the clinic, and both white and Negro patients who broke appointments.

In addition to class position, racial background was related to the reported duration of a disease. Twice as many Negro patients (34.3%) as white patients (16.7%) at the clinic indicated that they had had their disorders for less than two weeks. White and Negro patients who broke appointments followed this trend. One-third of the Negroes, in contrast to one-fifth of the white patients who broke appointments, had disorders lasting less than fourteen days.

Patients who broke appointments did not differ significantly from patients attending the clinic in the length of their disorders. Comparable trends are noted for both groups, i.e., that social class and race are related to the reported duration of a disease.

6. *Source of Referral*

Comparable referral patterns characterized individuals who attended or did not attend the clinic. Over a quarter of the patients in the study (27.3%) said they made appointments at the clinic on their own intiative. Other sources of referral were the family physicians (16.8%), Visiting Nurse Association (2.2%), local schools (13.6%), other hospital departments (25.6%), and others (14.5%). Patterns of referral for clinic patients and appointment breakers did not vary significantly by class or racial background.

7. *Reasons for Broken Appointments*

The 77 appointment breakers interviewed in their homes were asked for their opinions, first, about the clinic and secondly, why they broke their appointments. Approximately 60 per cent of all class groups had favorable opinions or no complaints about the clinic. Of those who did complain, twice as many upper class patients (24.0%) as lower class patients (12.5%) felt they "had to wait too long" for the physician. Analysis by race revealed that 82 per cent of the Negroes and 53 per cent of the Whites had no complaints about the Department. Again, most of the dissatisfaction about the clinic's service stemmed from the contention that the patients had to wait too long to receive treatment.

An informal spot check for a week revealed that a patient's waiting period in the clinic varied with the time of day when an appointment had been given. The waiting period for patients having appointments during the first half of the clinic's daily schedule averaged 15 minutes. In contrast, the average waiting period for patients during the second half of the clinic's daily schedule was 25 minutes.

The response which patients gave when asked why they broke their appointments were divided into "personal" and "other" reasons. Regardless of racial and class backgrounds or when an appointment had been given, 75 per cent of the appointments were broken because of "personal" reasons. The most frequent personal reasons given were "out of town," "child or sibling sick" or "poor weather." Other opinions, comprising 25 per cent of the responses, included "forgot," "no appointment slip given by the Department," "confusion about the date of the appointment," and "waiting too long for treatment."

DISCUSSION

Activity studies of hospital personnel have emphasized the importance of time in the co-ordination of hospital duties. Time is valuable and such stereotypes as "the busy doctor" or "the efficient nurse" epitomize for the layman this concern by medical personnel with the efficient use of available time. However, hospital workers make two implicit assumptions about time. The first assumption is that hospital time is more valuable than patient time, and secondly, that the concept of time held by patients and hospital employees is comparable. The first assumption has been challenged by Welch and Bailey who have compared the monetary value of the physician's time with the waiting periods of patients.[8]

Little information is available about the concepts of time which guide patients and hospital personnel. The data in this study of broken appointments would suggest that Negroes and lower class whites may adhere to different values of time than either those held by the other patients who were studied or the temporal organization of the Pediatric Out-Patient Department. Werner contends that the "time-binding" attributes of adult roles are learnt in childhood.[17] If this is the case, then the efficient utilization of time and being punctual may well be middle class white values which are not shared by lower class whites or Negroes.

These different concepts of time have implications for the organization of an outpatient department and the process of referral to such a unit. If an appointment system is being used, then the receptionist and other medical personnel should emphasize to the patients the importance of notifying the clinic if an appointment cannot be kept. Likewise, agencies and individuals who refer patients to a clinic could be requested to inform the patient about the organization of the clinic and the necessity of not breaking appointments. In this study 72.7 per cent of the patients who broke appointments had been referred to the Pediatric Out-Patient Department by community organizations, physicians, or other hospital departments. Greater co-opera-

tion between these sources of referral and the clinic might well reduce the number of broken appointments. In addition, since "waiting too long" was one of the main complaints of appointment breakers in this study, then it also may behoove clinic personnel to be punctual and to reduce the waiting period as much as possible.

SUMMARY

From December 1957 to March 1958 a total of 774 appointments given by the Pediatric Out-Patient Department of the Grace-New Haven Hospital were analyzed. Appointment-breakers, in contrast to patients who attended the clinic, were predominantly lower class whites, Negroes, and parents with very young children. Usual weather conditions, type of complaint or disease, length or disease, and source of referral were not related to the number of broken appointments. "Home" or personal reasons were given 75 per cent of the time as the reported cause of the broken appointments.

REFERENCES

1. Forsberg, R. and Lemal, J. M.: Why outpatients feel like outcasts. *Mod. Hosp.*, 1956, *86*, 94-102.
2. Welch, J. D. and Bailey, N. T. J.: Appointment systems in hospital outpatient departments. *Lancet*, 1952, *262*, 1105-1108.
3. Wilmot, I. G.: Enter: The ambulatory patient. *Hospitals*, 1958, *32*, 43 *et passim*.
4. Yvonne, M.: OPD appointment system. *Hosp. Progr.*, 1953, *34*, 61-62.
5. Hansen, A. C.: Broken appointments in a child health conference. *Nursing Outlook*, 1953, *1*, 417-419.
6. Olencki, M. and Reader, G. G.: Appointment breaking in a medical clinic. Paper read at American Public Health Association Convention, Atlantic City, N. J., October 19-23, 1959.
7. Badgley, R. F.: Social bias in the treatment of pediatric patients. *Pediatrics*, 1961, *27*, 829-835.
8. Furnal, M. A.: *Pediatric clinic appointment breaking.* New Haven, Yale University M.S. Thesis (unpublished), 1958.
9. Senn, M. J. E.: The changing role of a children's hospital. *Canad. M. A. J.*, 1957, *77*, 647-655.
10. Solnit, A. J. and Senn, M. J. E.: Teaching comprehensive pediatrics in an outpatient clinic. *Pediatrics*, 1954, *14*, 547-556.
11. Stark, M. H.: A social worker as member of a medical teaching staff. *Social Casework*, 1954, *35*, 245-252.
12. Davie, M. R.: The pattern of urban growth in *Studies in the Science of Society*, Murdock, G. P. (editor), New Haven, Yale University Press, 1937, pp. 133-161.
13. Hollingshead, A. B. and Redlich, F. C.: *Social class and mental illness.* New York, Wiley and Sons, Inc., 1958, pp. 47-136.
14. Myers, J. K.: Assimilation to the ecological and social systems of a community. *Amer. Sociol. Rev.*, 1950, *5*, 367-372.
15. Hollingshead, A. B.: *Two factor index of social position*, New Haven, Department of Sociology, Yale University, 1957.
16. National Conference on Medical Nomenclature: *Standard Nomenclature of Diseases and Operations.* New York, Blakiston Co., 1952.
17. Werner, H.: *Comparative psychology of mental development.* New York, Follett, 1948.

GERALD KLATSKIN*

Department of Internal Medicine, Yale University School of Medicine

THE EFFECT OF ETHYL ALCOHOL ON NITROGEN EXCRETION IN THE RAT**

In the course of investigating the effects of alcohol† on the liver of the rat it was found that alcohol increased the choline requirement, an effect that was independent of changes in food consumption or caloric intake.[1,2] To explore the possibility that alcohol induced a relative deficiency of choline by altering some aspect of protein metabolism, an investigation of the effects of alcohol on nitrogen balance was undertaken, the results of which are the subject of this report.

Although the rat has been used extensively to investigate the metabolism and nutritional value of alcohol, surprisingly few studies in this species have dealt with the problem of the effects of alcohol on nitrogen metabolism. Mitchell[3] observed that rats maintained on a liberal milk diet and given supplements of alcohol for a period of 42 days had a significantly higher carcass content of nitrogen than pair-fed unsupplemented controls. From this he concluded that the energy of alcohol is to a large extent available "for physiological purposes" and, thus, promotes growth and nitrogen retention. Although alcohol appeared to facilitate the digestion and absorption of dietary protein in these experiments, since it reduced losses of nitrogen in the feces, the calculated amount of nitrogen thus gained constituted only a small fraction of the extra nitrogen recovered in the carcass, so that it must be assumed that alcohol also reduced losses of nitrogen in the urine. In a more acute experiment, Shapiro[4] found that in rats subjected to fasting, the administration of alcohol for a period of four days failed to spare nitrogen and, indeed, in half the animals increased nitrogen losses in the urine.

Similarly variable results have been reported in the dog. In short-term experiments, Munk[5] found that small amounts of alcohol spared nitrogen while large amounts increased urinary nitrogen excretion. These observa-

---

* David P. Smith Professor of Medicine.

** Supported by a research grant (A-714) from the National Institutes of Health, United States Public Health Service.

† The term alcohol, as used in this paper, refers to ethyl alcohol.

Received for publication 15 May 1961.

tions were confirmed by Mendel.[6] However, Chittenden[7] reported that comparably large doses of alcohol spared nitrogen.

The results of studies in man have been equally contradictory, attributable, at least in part, to differences in the amount of alcohol administered, the state of nitrogen balance on the basal diet, and whether alcohol was administered as a supplement to the diet or as replacement for dietary carbohydrate and fat. In 1901 Rosemann[8] reported that alcohol spared nitrogen as effectively as carbohydrate and fat. Soon thereafter Atwater and Benedict[9] confirmed this observation, but pointed out that in certain susceptible individuals alcohol, by some pharmacological action, stimulated protein catabolism resulting in a transient increase in urinary nitrogen. In addition, they observed that alcohol reduced fecal nitrogen, an effect they attributed to more effective digestion and absorption of dietary protein. More recently, Rice and his associates[10] found that in surgical patients maintained in slightly positive nitrogen balance by parenteral feedings, alcohol supplements enhanced nitrogen retention, but that when alcohol was substituted for glucose isodynamically, the loss of nitrogen in the urine increased, resulting in negative nitrogen balance. In contrast, Summerskill, *et al.*[11] reported that in a group of alcoholics with cirrhosis, in nitrogen equilibrium or in slight positive balance, alcohol had no significant effect on nitrogen balance when it was given either as a supplement to the diet or as an isodynamic substitute for dietary carbohydrate.

In the present investigation of the effects of alcohol on nitrogen metabolism in the rat, two types of experiment were carried out. In the first, animals receiving alcohol and food *ad libitum* were compared with pair-fed controls given an isocaloric equivalent of sucrose instead of alcohol, and in the second, the animals were tube-fed during alternating periods with and without alcohol under several different dietary conditions.

PAIR-FEEDING EXPERIMENT

MATERIAL AND METHODS

Eight male Sprague-Dawley strain rats raised from weaning on Purina Fox Chow diet containing 20 per cent protein were transferred to individual metabolism cages when they had attained a weight of approximately 350 gm. The cages, which were housed in an air-conditioned room, maintained at a constant temperature of 23° C., and a humidity of 40-50 per cent, were designed to permit accurate, separate collections of urine and feces and to minimize coprophagy. Removable food cups were located outside the cage and were accessible to the animals only through a narrow, inverted L-shaped metal tunnel, an arrangement which effectively prevented food spillage.

The experimental diet contained vitamin-free casein (Labco) 12% by weight, Crisco 20%, corn oil 5%, sucrose 57.8%, cystine 0.2%, and salts IV 5%, and had a caloric value of 5.05 calories per gram. Vitamin supplements per 100 gm. of diet

included vitamin B_1, 1 mg.; vitamin B_{12}, 1 mg.; vitamin B_6, 1 mg.; nicotinic acid, 10 mg.; pantothenic acid, 10 mg.; biotin, 100γ; folic acid, 100γ; inositol, 20 mg.; paraminobenzoic acid, 29 mg.; vitamin K, 500γ; alpha-tocopheryl acetate, 10 mg.; vitamin D_2, 10γ; and vitamin A, 300 i.u. On direct Kjeldahl analysis the diet was found to contain 17.4 mg. of nitrogen per gm.

The animals were grouped into four pairs, one animal of each pair receiving the experimental diet *ad libitum*, the other serving as a pair-fed control. Measurement of food consumption and pair-feeding were carried out on a daily basis, a preliminary period of four days being allowed for equalization of food intake. In all but three of the subsequent 28 experimental periods of four days each, the pair-fed controls consumed more than 96 per cent of the diet offered. The exceptions are indicated in Table 1.

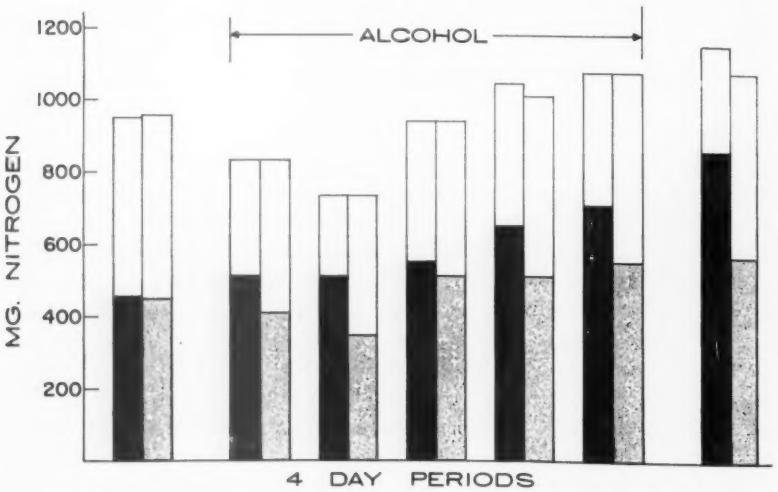


FIG. 1. Comparison of urinary nitrogen excretion in rat Aa receiving alcohol *ad libitum* (solid bar) with that of its pair-fed isocaloric control Ac (shaded bar). Nitrogen intake is indicated by the height of open bars.

Urine and feces were collected daily and analyzed for nitrogen in duplicate by the micro-Kjeldahl method of Willets and Ogg.[12] The wire-mesh floor of the cage, the glass collecting funnel, and the glass fecal deflector were thoroughly rinsed with distilled water bringing the urine to a constant volume of 50 ml. Toluene was used as a urinary preservative. Daily collections of urine and feces were stored in tightly sealed individual glass containers at minus 20° C. prior to analysis.

Following a control period of four days, a 15 per cent aqueous dilution of 95 per cent ethyl alcohol was substituted for the drinking water of the four experimental animals. The solution, which was dispensed in Richter tubes[13] to prevent significant losses by evaporation and spillage, contained 0.113 gm. of absolute alcohol and had a caloric value of 0.79 per ml., a figure based on the assumption that ethanol has a

caloric value of 7 calories per gm. Alcohol consumption was measured daily. To maintain isocaloric conditions, a caloric equivalent of sucrose (0.2 gm. per ml. of alcohol solution) was added to the diet of each pair-fed control. Alcohol was administered for 21 days, following which pair-feeding without alcohol was resumed for five days.

TABLE 1. COMPARISON OF AVERAGE INTAKE AND EXCRETION OF NITROGEN*

	Alcohol feeding						
Four-day periods	*1*	*2*	*3*	*4*	*5*	*6*	*7*
N intake, mg.							
Alcoholics	945	853	822	1004	931	909	1033
Controls	945	853	818	992	922	905	1027
Urine N, mg.							
Alcoholics	446	519	504	578	578	605	678
Controls	435	397	404	468	443	458	612
Fecal N, mg.							
Alcoholics	75	65	55	62	64	63	60
Controls	69	68	61	68	65	73	70
N balance, mg.							
Alcoholics	+424	+269	+263	+363	+289	+241	+295
Controls	+441	+387	+353	+456	+414	+373	+345
Food and alcohol intake in alcoholic group							
Food, gm.	54	49	48	58	53	52	59
Food, calories	273	247	242	293	268	263	298
Alcohol, ml.	0	61	62	74	65	66	0
Alcohol, calories	0	48	49	58	51	52	0
Total calories	273	295	291	351	319	315	298
Per cent alcohol calories	0	16	16	16	16	17	0

* Data from four rats fed on 15 per cent alcohol in lieu of drinking water is contrasted with data from their respective pair-fed isocaloric controls.
In calculating these averages the data for 3 of the 28 four-day experimental periods have been omitted because the pair-fed control in each instance failed to consume more than 96 per cent of the diet offered (Pair C animals, Period 1; Pair D animals, Period 4; Pair A animals, Period 7).

To facilitate analysis the daily data on nitrogen balance have been consolidated into four-day periods, i.e. one pre-alcohol, five alcohol, and one post-alcohol for each pair of animals. Data for the first day following cessation of alcohol administration have been omitted from consideration to exclude effects attributable to residual alcohol not yet eliminated.

RESULTS

The results, which were essentially the same in each pair of animals, are summarized in Table 1. Except as indicated in the footnote, the data tabulated represent average values for four animals in each of the seven collection periods of four days. Figure 1 illustrates the effect of alcohol on urinary nitrogen excretion in one representative pair of animals.

Urinary excretion of nitrogen rose above control values in all but one of the twenty periods of alcohol administration. In fifteen of these, urinary nitrogen increased despite a reduction in nitrogen intake. That alcohol was responsible for this effect is supported by the observation that in fifteen periods in which perfect equalization of food intake was achieved, the animals receiving alcohol invariably excreted more nitrogen than their pair-fed isocaloric controls. The increment in urinary nitrogen was evident throughout the twenty-day period of alcohol administration, and showed no consistent variation in magnitude.

Following withdrawal of alcohol, both the intake and urinary excretion of nitrogen increased. In two of the rats that had received alcohol, urinary nitrogen exceeded the control values of Period 1 despite a lower nitrogen intake in one and a negligibly higher intake in the other. Moreover, in two of the three pairs of rats in which equalization of food intake was achieved during post-alcohol Period 7, the rats previously given alcohol excreted more nitrogen than their pair-fed isocaloric controls. Although not conclusive, these data suggest that the effect of alcohol on nitrogen excretion may have been sustained for at least a brief period following alcohol withdrawal.

Fecal nitrogen excretion fell slightly below basal levels in all four animals given alcohol and remained lower than that found in their pair-fed controls.

All animals remained in positive nitrogen balance and gained weight throughout the course of the experiment, but the calculated amount of nitrogen retained diminished during periods of alcohol administration.

TUBE-FEEDING EXPERIMENT

MATERIAL AND METHODS

Six rats, weighing between 350 and 400 gm. each, were housed in individual metabolism cages, urine and feces being collected daily as described in the preceding experiment. Prior to analysis, the excreta for successive three-day intervals were pooled and are hereafter designated as "urine or fecal collections." There were ten such three-day collections in the preliminary period of observation, and four or five in each of the ten subsequent experimental and control periods.

Urine samples were analyzed in duplicate for: (i) total nitrogen by the micro-Kjeldahl method of Willets and Ogg,[12] (ii) creatinine nitrogen by a modification of the Hare method,[14] (iii) urea nitrogen by the ammonia microdiffusion method of Seligson[15] following treatment with urease, (iv) ammonia nitrogen by the Seligson method,[15] (v) alpha amino nitrogen by the method of Albanese and Irby,[16] (vi) semi-quantitative amino acid distribution by a modification of the Dent filter-paper chromatographic method,[17] and (vii) protein nitrogen by the micro-Kjeldahl method[12]

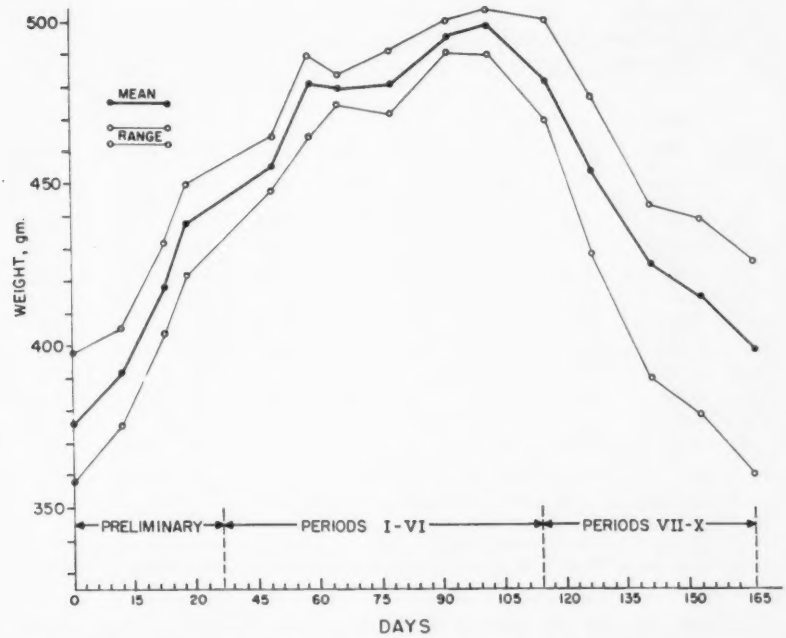


FIG. 2. Growth curves of tube-fed rats in Experient 2. The heavy line indicates the mean weight, the light lines the range of weights in five rats.

following precipitation with 5 per cent trichloracetic acid. Fecal nitrogen was determined in duplicate by the micro-Kjeldahl method.[12]

The animals were fed twice daily, at 9:30 A.M. and 3:30 P.M., by intubating the stomach with a Davol No. 8 French polyethylene infant-feeding tube, and slowly instilling food, or food and alcohol, as described below, with a calibrated 10 ml. syringe. To permit complete delivery of the volume expelled from the syringe without having to empty the feeding tube with air or additional water, the syringe was filled with the appropriate volume of the feeding mixture with the tube attached. Before starting the experiment, all animals were adapted to tube-feeding by instilling the experimental diet twice daily in increasing volumes over a two-week period. Early in the experiment one animal died suddenly as a result of regurgitation and aspiration

of the intubation mixture, so that the results reported are based on the data obtained in the remaining five animals. One animal developed diarrhea in Period 7, so that the data for this and all subsequent periods in this animal were discarded. The remaining animals tolerated the tube-feedings remarkably well, and remained in apparent good health and without diarrhea throughout the experiment. As indicated in Figure 2, all animals gained weight at a satisfactory rate when given an amount of food equal to that consumed by a previously studied group of *ad libitum*-fed rats on the same diet (Periods 1-6), but lost weight when this amount was halved (Periods 7-10).

TABLE 2. COMPOSITION OF TUBE FEEDINGS PREPARED DAILY

					Total daily ration per rat				
Period	*Basal diet*	*15 per cent*			*Volume*	*Weight*	*Diet content*	*Alcohol content*	*N content*
		Water	*alcohol*	*Sucrose*					
	gm.	*ml.*	*ml.*	*gm.*	*ml.*	*gm.*	*gm.*	*gm.*	*mg.*
1	140*	87.9	0	0	20	22.8	14	0	240.8
2	140*	54.6	33.3	0	20	22.8	14	2.498	240.8
3	140	87.9	0	0	20	22.8	14	0	240.8
4	140	54.6	33.3	0	20	22.8	14	2.498	240.8
5	140	87.9	0	0	20	22.8	14	0	240.8
6	96.3**	98.3	33.3	0	20	22.8	14	2.498	240.8
7	70*	44.2	0	43.7	13.8	15.8	7	0	120.4
8	70*	54.6	33.3	0	13.8	15.8	7	2.498	120.4
9	70*	44.2	0	43.7	13.8	15.8	7	0	120.4
10	140*	87.9	***	0	10	11.4	7	***	120.4

* Supplemented with 0.5 per cent choline hydrochloride.
** Special diet; see text.
*** Alcohol not incorporated in diet; 10.6 ml. of 30 per cent solution of alcohol, equivalent to 2.498 gm. of absolute alcohol, given seven hours after diet-feeding.

The basal diet was the same as in the preceding experiment. To permit the isocaloric substitution of alcohol for sucrose in the basal diet in Period 6, a special diet with the following composition was used: casein 17.4%, Crisco 29.1%, vitaminized corn oil 7.3%, vitaminized sucrose 7.3%, sucrose 32%, cystine 0.3% and salts IV 7.3%. When administered as described below, this diet provided identical amounts of protein and fat and the same number of calories as in Periods 1, 3, and 5.

The feeding mixtures of diet and water, or diet, water, and alcohol were made up daily, homogenized in a Waring blendor, and refrigerated between feedings. In Periods 1-6 the prescribed amount of diet per feeding was administered in a total volume of 10 ml., and in Periods 7-10 in a total volume of 6.9 ml. The volume of water in each feeding was held constant, the discrepancy between the feeding-mixture volumes administered in the first and second half of the experiment being due to a difference in the amount of diet in the mixtures.

The amount of water required to yield the correct volume after homogenization of any given feeding mixture was determined by trial and error (Table 2). After

establishing the weight of an aliquot of the mixture, the constancy and uniformity of the mixture were checked daily by weighing random samples. Usually the deviations from the theoretical were less than ±1.0 per cent and never exceeded ±2.5 per cent. As a further check, the mixtures were analyzed for their nitrogen content. Similarly small deviations from expected values were found.

Before undertaking a detailed investigation of nitrogen balance, a preliminary study of urinary nitrogen and creatinine excretion was carried out to ensure that the animals were adapted to the tube-feeding regimen and were excreting nitrogen at a constant rate. Considering the fact that the animals had been tube-fed for two weeks before the experiment began, that

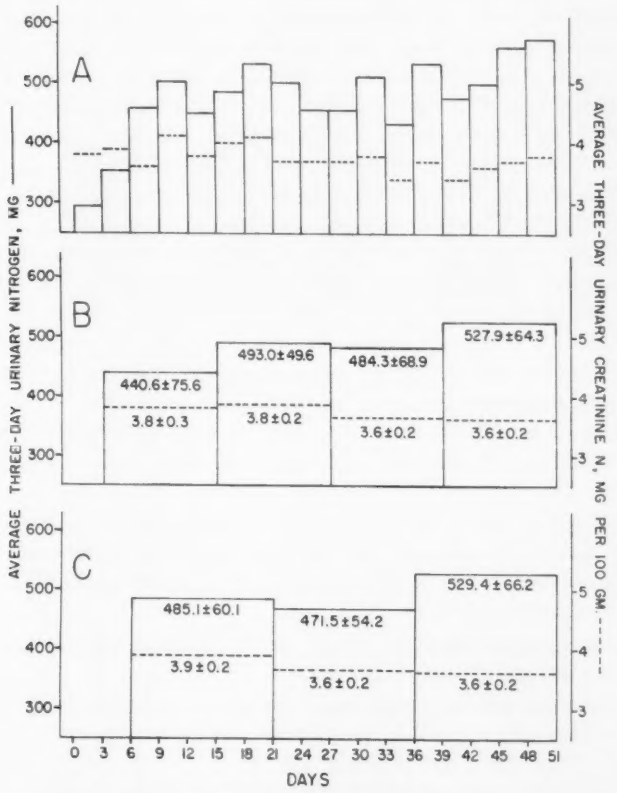


FIG. 3. Urinary excretion of nitrogen and creatinine of five tube-fed rats during the preliminary study period. Panel A shows the mean values for successive individual three-day collections, Panel B the mean values for four successive three-day collections, and Panel C the mean values for five successive three-day collections.

the nitrogen intake was fixed, and that the environmental conditions and methods of separating and collecting the excreta were rigidly controlled, the fluctuations in the nitrogen content of successive three-day collections of urine were unexpected (Fig. 3, Panel A). Creatinine excretion, expressed in terms of body weight, was less erratic, suggesting that the fluctuations in nitrogen excretion were not due to faulty technique in collecting the urine. To exclude the possibility that they were related to variations in the voluntary consumption of water, which was freely available to the animals through the first fifteen days of the preliminary study, the amount of water, including that present in the tube-feedings, was, for the remainder of the experimental period, limited to 24 ml. per day, a volume that was selected on the basis of the average voluntary intake in a group of rats receiving the same diet *ad libitum*. Since this failed to stabilize the rate of nitrogen excretion, the possibility was considered that it might be feasible to establish a constant pattern of nitrogen excretion and to demonstrate changes induced by alcohol, by pooling the values obtained for all animals over a longer period of time, and by treating the data statistically. Figure 3, Panel B gives such pooled data for three successive twelve-day periods. The values indicated in the upper segment of each block represent the mean and standard deviation for the 20 nitrogen determinations obtained on 4 three-day collections in each of five rats. It will be noted that the pooled values for successive twelve-day periods did not differ significantly. As shown in Panel C of Figure 3, the same was true for the pooled data for successive fifteen-day periods based on the 25 determinations obtained on 5 three-day collections in each of five rats for each period.

In both instances creatinine excretion showed very small but statistically significant differences. Assuming that these were attributable to losses of urine during collection, the calculated values for total nitrogen corrected for such losses still showed no statistically significant differences in successive twelve-day or fifteen-day periods. On the basis of these preliminary observations, all data in subsequent experimental periods were treated in a similar manner.

The experimental design is shown schematically in Figure 4, and may be summarized as follows:

1. *Control Period* (15 days): basal diet with 0.5 per cent choline, 14 gm. daily, containing 70.7 calories.

2. *Alcohol Period* (15 days): basal diet with choline as in Period 1 *plus* 2.498 gm. absolute alcohol (one half included in each of the two 10 ml. feedings) providing 17.5 calories, or 19.8 per cent of the total intake of 88.2 calories.

3. *Control Period* (12 days): same conditions as in Period 1, except that the basal diet contained no added choline.

4. *Alcohol Period* (12 days): same conditions as in Period 2 except that the basal diet contained no added choline.

5. *Control Period* (12 days): same conditions as in Period 3.

6. *Alcohol Period* (12 days): basal diet without added choline, 14 gm. daily, with 2.498 gm. absolute alcohol *substituted* for an isocaloric equivalent of sucrose, yielding an intake of 70.7 calories, as in control Periods 1, 3, and 5.

7. *Control Period* (12 days): basal diet with 0.5 per cent choline, 7 gm. daily (35.4 calories), plus 4.37 gm. sucrose (calorically equivalent to 2.498 gm. of absolute

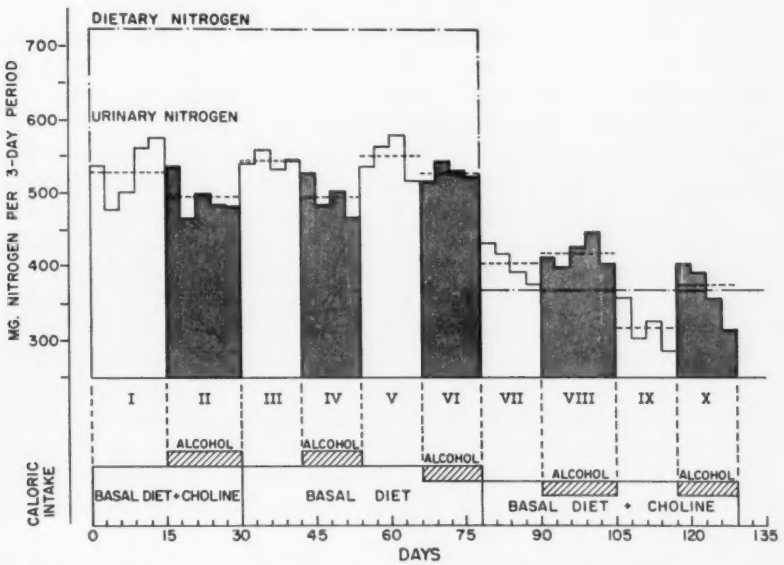


FIG. 4. Effect of alcohol on urinary nitrogen excretion in tube-fed rats under varying dietary conditions. The mean values for individual three-day collections in five rats are shown by the solid lines at the head of each column, the mean values for successive three-day collections in each experimental period by the broken lines. Solid bars indicate periods during which alcohol was administered.

alcohol administered in Periods 2, 4, 6, 8, and 10), yielding a total intake of 52.8 calories.

8. *Alcohol Period* (15 days): conditions as in Period 7 except that 2.498 gm. absolute alcohol was *substituted* isocalorically for the 4.37 gm. sucrose supplement in that period.

9. *Control Period* (12 days): conditions as in Period 7.

10. *Alcohol Period* (12 days): conditions as in Period 8 except that the 7 gm. of basal diet was given in one feeding at 9:30 A.M., while the alcohol was administered in one dose at 4:30 P.M.

RESULTS

The data obtained are summarized in Table 3.

Urinary Nitrogen. With the animals on a high intake of protein and calories, adding alcohol to the diet reduced losses of nitrogen in the urine, both when the diet contained a supplement of choline (Period 2) and when it did not (Period 4). In each instance the withdrawal of alcohol was followed by a prompt return of the urinary nitrogen to control levels (Periods 3 and 5). As illustrated in Figure 4, the nitrogen-sparing effect of alcohol was not apparent during the first three days of alcohol administration, but was sustained thereafter for twelve days in Period 2 and for nine days in Period 4. Substituting alcohol for sucrose with no net change in caloric intake in Period 6 had no effect on urinary nitrogen excretion.

On reducing the intake of protein and calories in Period 7 there was a sharp fall in urinary nitrogen. Substituting alcohol for sucrose in the following period resulted in a prompt increase in urinary nitrogen which was sustained (Fig. 4). Although the mean output for the entire period was not significantly greater than that of the preceding control period, it will be observed in Figure 4 that nitrogen excretion fell significantly when alcohol was withdrawn in Period 9, and then rose significantly when alcohol was administered for a second time in Period 10. As shown in Figure 4, urinary nitrogen excretion fell progressively during control Periods 7 and 9, possibly in relation to weight loss (Fig. 2) and decreased metabolic activity. It is highly probable that this trend partially obscured the effects of alcohol in Periods 8 and 10 thus, being responsible for the fact that the mean increase in urinary nitrogen in Period 8 was not statistically significant, and that the increase in nitrogen excretion in Period 10 was not sustained (Fig. 4).

Urinary Creatinine. The excretion of creatinine appeared to vary inversely with that of total nitrogen. Thus, when the animals were on a high intake of protein and calories, supplementing the diet with alcohol in Periods 2 and 4 led to a small but significant increase in urinary creatinine, while substituting alcohol for sucrose in Period 6 had no effect. In contrast, when the intake of protein and calories was sharply reduced, substituting alcohol for sucrose resulted in a small decrease in urinary creatinine. This was statistically significant in Period 8 but not in Period 10.

The significance of these fluctuations in creatinine excretion in terms of nitrogen metabolism is uncertain. However, even if they were attributable to variations in renal function or to inaccuracies in urine collection, their direction was such that they could not have accounted for the changes in total nitrogen excretion described above.

TABLE 3. THREE-DAY INTAKE AND OUTPUT OF NITROGEN IN TUBE-FED RATS

Except where otherwise indicated the values recorded are the means and standard deviations for all determinations on either four or five successive three-day collections in each of five animals in Periods 1-6 and in each of four animals in Periods 7-10. Statistically significant differences between successive values are indicated by asterisks (* $p < .05$, ** $p < .01$).

Period	Nitrogen intake	Urine nitrogen								Fecal‡ nitrogen	Nitrogen balance
		Total	Creatinine	Urea	Ammonia	Allantoin	Amino acid	Protein†	Undetermined		
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
1											
15 days	722.4	529.4±66.2	16.4±1.0	357.9±72.2	37.9±11.5	34.9±3.7	10.6±2.7	3.5±0.4	71.7±74.0	45.2	+147.8±66.2
2		*	**				**		**		
15 days	722.4	494.9±37.8	17.9±1.6	358.2±32.2	38.3±5.5	36.0±7.8	16.8±4.5	4.4±1.0	26.4±18.5	60.4	+167.1±37.8
3		**	**	**	**		**				**
12 days	722.4	544.9±42.6	16.9±1.3	399.1±34.6	25.6±5.2	35.8±3.6	13.5±4.1	4.7±0.8	49.3±53.2	79.7±12.1	+97.8±51.6
4		**	**	**							**
12 days	722.4	496.2±47.1	17.7±1.8	368.3±30.1	27.0±5.9	33.9±4.3	14.6±3.4	4.6±0.9	31.0±21.8	77.1±4.6	+149.1±44.9
5		**		**					**		**
12 days	722.4	550.1±44.2	17.2±1.5	394.0±23.9	25.7±4.8	34.3±2.5	13.2±4.7	5.5±1.4	60.2±28.0	77.2±7.5	+95.1±42.2
6				*						**	**
12 days	722.4	529.1±27.4	17.6±1.1	372.1±31.0	24.6±3.5	32.5±2.8	12.4±2.2	4.2±0.9	66.1±27.4	59.0±8.3	+134.2±31.1
7		**	**	**	*		**	*			**
12 days	361.2	404.9±30.7	16.3±1.0	246.9±28.7	21.8±3.5	34.7±3.7	8.8±2.4	2.5±0.4	73.9±25.3	49.8±7.7	—93.5±33.8
8			**		**	**		*		**	
15 days	361.2	418.4±64.7	14.5±1.2	254.2±41.8	28.3±3.5	25.2±3.1	9.2±2.2	1.5±0.5	85.5±41.8	29.9±1.0	—87.1±65.2
9		**		**	**		**			**	**
12 days	361.2	318.1±45.3	14.3±1.3	184.7±43.5	23.4±3.9	23.5±3.2	6.5±1.7	1.1±0.4	68.5±40.1	40.0±6.3	+3.1±45.6
10		**		**	**	*	**	**	**	**	
12 days	361.2	375.1±84.7	13.3±2.2	260.1±73.8	35.1±7.5	27.3±3.8	10.7±3.0	3.4±0.5	30.2±30.7	26.9±3.5	—40.3±85.2

† Urine specimens for each animal were pooled, so that the values recorded represent the means and standard deviations of either four or five determinations.
‡ In periods 1 and 2 all feces were pooled; in Periods 3-10 the feces for each animal were pooled, so that the values recorded represent the means and standard deviations of either four or five determinations.

Urinary Urea. The effects of alcohol on urea excretion paralleled those on total nitrogen excretion, but were less consistent. Thus, when the animals were on a high intake of protein and calories, supplementing the diet with alcohol reduced the output of urea in Period 4, but not in Period 2. It is noteworthy, however, that when alcohol was omitted in Period 3, urea excretion increased significantly. Although substituting alcohol for sucrose in Period 6 had no effect on total urinary nitrogen, it resulted in a significant decrease in urea excretion.

As in the case of total nitrogen, urea exretion increased significantly when alcohol was substituted for sucrose under conditions of reduced nitrogen and caloric intake (Periods 8 and 10). Although the increase was not statistically significant in Period 8, the withdrawal of alcohol in the following period resulted in a significant decrease in urea excretion.

Urinary Ammonia. When the intake of nitrogen and calories was high, alcohol had no effect on ammonia excretion whether it was given as a supplement (Periods 2 and 4) or as a substitute for dietary carbohydrate, (Period 6). However, when the intake of protein and calories was curtailed, substituting alcohol for sucrose resulted in an increase in urinary ammonia, both in Period 8 and in Period 10.

Urinary Allantoin. During Periods 1 through 6, when nitrogen intake was relatively high, allantoin excretion remained remarkably constant, and was not affected by alcohol administration. However, it fell significantly in Period 8 and remained low thereafter. Although the decrease in allantoin excretion coincided with the administration of alcohol in Period 8, the fact that there was no increase when alcohol was withdrawn in Period 9, or fall when alcohol was given a second time in Period 10, suggests that the decline in allantoin excretion was related to the reduction in food intake that preceded it.

Urinary Amino Nitrogen. The effects of alcohol on urinary amino nitrogen excretion were inconstant. Thus, amino nitrogen increased significantly when alcohol was administered in Periods 2 and 10, but not in Periods 4, 6, and 8.

As indicated in Table 4, the pattern of amino acids excreted was relatively constant throughout the experiment, and was not affected by alcohol. No methionine was detectable in the urine during any of the experimental periods. Since the chromatographic method of analysis employed was sensitive to 5 μg in a 150 μl spot, it must be assumed that if any methionine was excreted it was in amounts of less than 5 mg. per three-day period.

Urinary Protein. Qualitative tests for albumin were negative in all urine samples collected. However, micro-Kjeldahl analysis of trichloracetic acid

precipitates of pooled urines from each of the animals revealed the presence of a small but significant fraction of protein nitrogen in each. The amount excreted remained relatively constant during Periods 1 through 6, but fell significantly when the intake of nitrogen and calories was reduced in Period 7. Under these dietary conditions, substituting alcohol for sucrose had inconstant effects on urinary protein, lowering the excretion in Period 8 and raising it in Period 10.

TABLE 4. SEMI-QUANTITATIVE ESTIMATE OF THREE-DAY AMINO ACID EXCRETION BASED ON PAPER CHROMATOGRAPHIC ANALYSIS OF POOLED URINE COLLECTED IN EACH PERIOD

The ninhydrin-stained spots obtained with 150 μl aliuots were compared with those given by standard solutions of amino acid.

	Periods					
	1	*2*	*5*	*3*	*4*	*6*
Taurine, mg.	35	25	35	35	30	30
Cystine, mg.	Trace	Trace	Trace	4	Trace	5
Alanine, mg.	Trace	Trace	Trace	Trace	Trace	Trace
Serine, mg.	Trace	Trace	Trace	Trace	Trace	0
Glycine, mg.	Trace	Trace	Trace	Trace	Trace	0
Glutamic acid, mg.	4	4	4	5	4	5
Aspartic acid, mg.	0	0	0	Trace	0	0
Methionine, mg.	0	0	0	0	0	0

"Undetermined" Urinary Nitrogen. In all but a few urine samples, the total nitrogen determined by the micro-Kjeldahl method exceeded the sum of the nitrogen values for urea, ammonia, creatinine, allantoin, amino acids, and protein. The difference which was averaged as 5 to 21 per cent of the total nitrogen in different periods, exceeded the expected cumulative errors of analysis and suggested the presence in the urine of undetermined nitrogen-containing compounds. The nature of these compounds was not investigated further.

Under conditions of a high intake of protein and calories, the alcohol-induced changes in the excretion of "undetermined" nitrogen paralleled those of total urinary nitrogen. Thus, excretion fell when alcohol was added to the diet (Periods 2 and 4), but not when it was substituted for dietary sucrose (Period 6). Although the decrease in Period 4 was not statistically significant, there was a significant increase when alcohol was withdrawn in the following period.

The results of alcohol administration on "undetermined" nitrogen excretion under conditions of a low intake of protein and calories were in-

constant. Thus, substituting alcohol for sucrose had no effect in Period 8, but reduced the urinary losses of 'undetermined" nitrogen in Period 10.

Fecal Nitrogen. Alcohol reduced the losses of nitrogen in the feces in three of the four periods in which unpooled values were available for statistical analysis (Periods 6, 8, and 10). Thus, this effect was observed at both high and low levels of protein intake.

Nitrogen Balance. With a food intake of 14 gm. daily in Periods 1 through 6, all animals remained in positive nitrogen balance and gained

TABLE 5. NET CHANGES* IN NITROGEN EXCRETION INDUCED BY ALCOHOL ADMINISTRATION*

	Period				
	2	4	6	8	10
	mg.	mg.	mg.	mg.	mg.
Urine nitrogen					
Total	*−34.5*	*−48.7*	−21.0	+13.5	*+57.0*
Creatinine	*+1.5*	*+0.8*	+0.4	*−1.8*	−1.0
Urea	+0.3	*−30.8*	*−21.9*	*+7.3*	*+75.4*
Ammonia	+0.4	+1.4	−1.1	*+6.5*	*+11.7*
Allantoin	+1.1	−1.9	−1.8	*−9.5*	*+3.8*
Amino acid	*+6.2*	+1.1	−0.8	+0.4	*+4.2*
Protein	+0.9	−0.1	−1.3	*−1.0*	*+2.3*
"Undetermined"	*−45.3*	*−18.3*	+5.9	+11.6	*−38.3*
Fecal nitrogen	+15.2	−2.6	*−18.2*	*−18.9*	*−13.1*
Nitrogen balance	*+19.3*	*+51.7*	*+39.1*	+6.4	*−43.4*

* Each of the values reported represents the difference between the mean for all three-day determinations in any given period of alcohol administration and that found in the immediately preceding control period. Statistically significant differences are italicized.

weight at a relatively constant weight (Fig. 2). Under these conditions alcohol increased nitrogen storage both when it was administered as a supplement in Periods 2 and 4, and when it was substituted for sucrose in Period 6. Although the increase in Period 2 was not significant, there was a statistically significant decrease in nitrogen storage when alcohol was withdrawn in the following period.

When their food intake was reduced to 7 gm. daily, all animals lost weight and went into negative nitrogen balance (Periods 7-10). Substituting alcohol for sucrose in Periods 8 and 10 had no significant effect, but the withdrawal of alcohol in Period 9 resulted in a significant increase in nitrogen retention.

Effects of Alcohol on Partition of Urinary Nitrogen. As shown in Table 5, the net effect of alcohol on total urinary nitrogen excretion and nitrogen balance was determined principally by changes in urea excretion, except in Period 2 where the fall in urinary nitrogen and increase in positive nitrogen balance appeared to be related to a decrease in "undetermined" urinary nitrogen.

COMMENT

From the data presented it is evident that the effects of alcohol on nitrogen excretion in the rat are variable, and are influenced by several factors.

In Experiment 1, with the animals in positive nitrogen balance, *ad libitum* oral feeding of alcohol increased urinary losses of nitrogen despite a concomitant decrease in nitrogen intake. Yet, under similar dietary conditions* in Periods 2 and 4 of Experiment 2, when even larger amounts of alcohol** were tube-fed well mixed with the diet, urinary nitrogen excretion fell. It is reasonable to assume that this reversal of the action of alcohol from one of nitrogen-wasting in Experiment 1 to one of nitrogen-sparing in Experiment 2 was related to differences in experimental conditions. Of these the most important were: i) alcohol intake in Experiment 2 was approximately a third greater than in Experiment 1, ii) the intake of dietary nitrogen and calories fell below control values in Experiment I (Table 1), but remained constant in Periods 2 and 4 of Experiment 2, iii) although the total intake of calories, including alcohol calories, was increased above control levels in both experiments, the increment was greater in Experiment 2 than in Experiment I (Table 1), and iv) in Experiment I the animals had free access to food and alcohol at all times and ate and drank intermittently throughout the day, while in Experiment 2, food and alcohol were well mixed and administered by gastric tube twice daily. It is highly improbable that alcohol wastes nitrogen when given in small amounts, as in Experiment 1, and spares nitrogen when given in larger amounts, as in Experiment 2. The possibility that alcohol spares nitrogen only when a critical level of nitrogen and/or caloric intake is attained, and wastes nitrogen below this level, even though positive nitrogen balance is maintained, as in Experiment 1, and thus accounts for the discrepancy between the results of Experiment 1 and 2, appears more plausible. However, as can be seen in Table 1, alcohol increased urinary losses of nitrogen in Period 4

* The average daily intake of nitrogen was 226 mg. in Experiment 1 and 242 mg. in Experiment 2.

** The average daily intake of alcohol was 1.854 gm. in Experiment 1 and 2; 498 gm. in Experiment 2.

of Experiment 1, even though the intakes of nitrogen, dietary calories and total calories were above control levels and equal to those of Periods 2 and 4 of Experiment 2 in which alcohol reduced urinary losses of nitrogen. An alternative possibility to be considered is that alcohol supplements*** spare nitrogen under conditions of positive nitrogen balance when metabolized together with other nutriments, as in Periods 2 and 4 of Experiment 2, but enhance nitrogen losses when metabolized alone, as undoubtedly occurred under the conditions of *ad libitum* intermittent eating and drinking in Experiment 1. Some support for this possibility is provided by the observation that alcohol increased the urinary excretion of nitrogen to a greater degree in Period 10 when alcohol and food were administered separately seven hours apart, than during Period 8 when they were administered together (Table 5). Also consistent are the results obtained in previously reported studies in the rat. Thus, alcohol spared nitrogen when incorporated in the basal diet in Mitchell's experiments,[8] but failed to do so, and indeed, often increased urinary losses of nitrogen, when fed without food in Shapiro's experiments.[4]

Substituting alcohol for an isocaloric equivalent of sucrose in the diet had no effect in Period 6, but enhanced urinary losses of nitrogen in Periods 8 and 10 of Experiment 2. Since the animals were in positive nitrogen balance in Period 6 and in negative nitrogen balance in Periods 8 and 10, it is possible that the nitrogen-wasting effect of alcohol, ascribed by Atwater and Benedict[9] to an inconstant pharmacological action, was dependent, in part at least, upon a low dietary intake of protein. Unfortunately, the evidence on this point is not conclusive. While there was a definite increase in urinary nitrogen in Period 8 the mean for all determinations during the period was not significantly greater than that of the preceding control period. Also, in Period 10 the increase in urinary nitrogen could have been related to the fact that food and alcohol were administered separately seven hours apart, rather than that the dietary intake of nitrogen was low. One factor that may have minimized the nitrogen losses and thus obscured the nitrogen-wasting effect of alcohol in Period 8 was the tendency for the urinary output of nitrogen to decline between Periods 7 and 10, as evidenced by the striking difference between the mean values for control Periods 7 and 9 (405±31 and 318±45 mg., respectively). This probably reflected a

*** As can be seen in Table 1, alcohol lowered the food intake below control levels during the first two four-day collection periods of Experiment 1 and thus appeared to replace part of the diet. However, food intake returned to control levels during the three remaining four-day collection periods and total calorie intake was above control levels in all five of the four-day collection periods during alcohol administration, so that the alcohol was in this sense a dietary supplement.

decrease in the demand for nitrogen associated with the progressive weight loss that followed curtailment of the diet in Period 7. Taking this factor into account together with the observation that urinary nitrogen fell sharply in Period 9 when alcohol was withdrawn, it is highly probable that the increase in urinary nitrogen in Period 8 was significant, even if it was not demonstrable statistically. If such was the case, it would be reasonable to assume that this effect was dependent upon the low intake of protein and calories, since it was not observed in Period 6 when the intake was relatively high.

On analyzing the effects of alcohol on the partition of urinary nitrogen, no distinctive pattern of response on the part of any of the fractions other than urea can be discerned. It is difficult to account for the erratic behavior of these fractions considering that the intake of food, alcohol and water, and the environmental temperature and humidity were rigidly controlled, that the experimental periods were relatively long, and that great care and uniform techniques were employed in collecting and analyzing all specimens. One factor that could not be controlled was the degree of motor activity of the animals. Judging from daily observations, this did not appear to vary either spontaneously or under the influence of changes in diet or alcohol intake. However, this aspect of the problem merits further investigation.

As in Mitchell's study,[8] alcohol showed a tendency to reduce fecal losses of nitrogen. It is not clear whether this was due to more effective digestion and absorption of dietary protein, or to a decrease in the intestinal secretions. Also to be considered is the possibility that alcohol affected the extent to which coprophagy occurred, and thus influenced the fecal output of nitrogen. Although the animals in these experiments were housed in raised wire-mesh metabolic cages, it is well known that this conventional method of preventing coprophagy is ineffective.[18]

The data presented shed no light on the metabolic factors involved in alcohol-induced changes in nitrogen excretion. However, it is evident that they are not dependent upon the previously described relative deficiency of choline that alcohol induces,[1,3] since large supplements of choline neither affected nitrogen-sparing in Period 2 nor abolished nitrogen-wasting in Periods 8 and 10 of Experiment 2.

In a previous report[3] it was shown that in weanling rats it requires a choline supplement of 0.08 per cent of the diet to meet the increased demand for choline induced by alcohol. More recently it has been found that 3 mole equivalents of methionine are equally effective.[19] Accordingly, if alcohol induces a relative choline deficiency by increasing losses of methionine in the urine, the animals in Experiment 2 receiving 14 gm. of diet would have

had to excrete approximately 35 mg. of methionine daily to account for such a deficiency. Taking into account the possibility that the methionine requirement may be very much lower in adult rats than in weanlings and that the analytical method employed may have failed to detect traces of methionine, the fact that the amount excreted could not have been as much as 2 mg. per day makes it highly improbable that alcohol induces a relative deficiency of choline by increasing losses of methionine in the urine.

SUMMARY

The effects of alcohol on nitrogen excretion in the rat have been investigated in animals on an *ad-libitum* intake of food and 15 per cent alcohol, on pair-fed animals given an isocaloric equivalent of sucrose in lieu of alcohol serving as controls, and also in tube-fed animals given food alone or food mixed with alcohol in alternating periods under varying dietary conditions. The data obtained suggest that supplementing the diet with alcohol spares nitrogen by reducing urinary losses of nitrogen, especially urea when the alcohol is tube-fed together with food, but increases urinary losses of nitrogen when the alcohol and food are ingested intermittently and presumably separately. The data also suggest that substituting alcohol for an isocaloric equivalent of sucrose in the diet has no effect under conditions of positive nitrogen balance, but increases nitrogen losses in the urine under conditions of negative nitrogen balance. In addition, alcohol tends to reduce fecal losses of nitrogen. The effects of alcohol on motor activity and coprophagy are two possible complicating factors not controlled in these experiments, and they require further study.

REFERENCES

1. Klatskin, G., Krehl, W. A., and Conn, H. O.: The effect of alcohol on the choline requirement. I. Changes in the rat's liver following prolonged ingestion of alcohol. *J. exp. Med.*, 1954, *100*, 605.
2. Klatskin, G. and Krehl, W. A.: The effect of alcohol on the choline requirement. II. Incidence of renal necrosis in weanling rats following short-term ingestion of alcohol. *J. exp. Med.*, 1954, *100*, 615.
3. Mitchell, H. H.: The food value of ethyl alcohol. *J. Nutr.*, 1935, *10*, 311.
4. Shapiro, I.: Studies on ketosis. I. The comparative glycogenic and ketolytic action of glucose and some carbohydrate equivalents. *J. biol. Chem.*, 1935, *108*, 373.
5. Munk, I.: Cited by Rosemann, R., *Pflüg. Arch. ges. Physiol.*, 1901, *86*, 307 (see ref. 8).
6. Mendel, L. B. and Hilditch, W. W.: The influence of alcohol upon nitrogenous metabolism in men and animals. *Amer. J. Physiol.*, 1910, *27*, 1.
7. Chittenden, R. H.: The influence of alcohol on proteid metabolism. *J. Physiol.*, 1891, *12*, 220.
8. Rosemann, R.: Der Einfluss des Alkohols auf den Eiweissstoffswechsel. *Pflüg. Arch. ges. Physiol.*, 1901, *86*, 307.

9. Atwater, W. O. and Benedict, F. G.: An experimental inquiry regarding the nutritive value of alcohol. *Mem. Natl. Acad. Sci.,* 1902, *8,* 231.
10. Rice, C. O., Orr, B., and Enquist, I.: Parenteral nutrition in the surgical patient as provided from glucose, amino acids, and alcohol. The role played by alcohol. *Ann. Surg.,* 1950, *131,* 289.
11. Summerskill, W. H. J., Wolfe, S. J., and Davidson, C. S.: Response to alcohol in chronic alcoholics with liver disease. Clinical, pathological, and metabolic changes. *Lancet,* 1957, *1,* 335.
12. Willets, C. O. and Ogg, C. L.: Report on standardization of microchemical methods. Micro Kjeldahl nitrogen determination. *Assn. Official Agr. Chem.,* 1950, *33,* 179.
13. Richter, C. P.: A study of the effect of moderate doses of alcohol on the growth and behavior of the rat. *J. exp. Zool.,* 1926, *44,* 397.
14. Hare, R. S.: Endogenous creatinine in serum and urine. *Proc. Soc. exp. Biol. (N. Y.),* 1950, *74,* 148.
15. Seligson, D. H. and Seligson, H.: A microdiffusion method for the determination of nitrogen liberated as ammonia. *J. Lab. clin. Med.,* 1951, *38,* 324.
16. Albanese, A. A. and Irby, V.: Determination of urinary amino nitrogen by the copper method. *J. biol. Chem.,* 1944, *153,* 583.
17. Dent, C. E.: A study of the behavior of some sixty amino-acids and other ninhydrin-reacting substances on phenol-'collidine' filter-paper chromatograms, with notes as to the occurrences of some of them in biological fluids. *Biochem. J.,* 1948, *43,* 169.
18. Barnes, R. H., Fiala, G., McGehee, B., and Brown, A.: Prevention of coprophagy in the rat. *J. Nutr.,* 1957, *63,* 489.
19. Klatskin, G.: Unpublished data.

LEO H. VON EULER*

Department of Pathology, Yale University School of Medicine

MESONEPHRIC DUCT CYST IN A NEWBORN

In the female embryo large portions of the mesonephric duct degenerate before birth,[1] whereas in the male, the duct remains and is incorporated into the genital system. That which remains in the female forms parts of the urethra and trigone, and contributes to the epoophoron which persists through life. Occasionally the duct remains in the lateral wall of the vagina and uterus as Gärtner's duct. All these remnants are subject to cystic and neoplastic change, and it is usually as a result of such pathology that they are identified and studied.

The origin of cysts in the vicinity of the kidneys has not been clear, but has not been ascribed to remnants of the mesonephric duct. Some observers, however, have described cysts which they have identified as mesonephric rests.[2-8] Most of these cysts have had no anatomic relationship to any other organ but the kidney, and the cavity has generally been blind, without communications with the renal pelvis. The present report concerns a large retroperitoneal cyst in continuity with the right kidney and the right side of the uterine cervix.

CASE REPORT

The patient, a Negro female infant (autopsy #14376), was the issue of an uneventful pregnancy. The mother was a 33-year-old woman whose serology was negative and whose blood type was Rh positive. Onset of labor and rupture of membranes were spontaneous. Total duration of labor was 1½ hours, with the second stage described as precipitous. The 5-minute Apgar rating was 8, and some cyanosis was present. Birth weight was 1900 gm. On physical examination the eyes were thought to have an Oriental slant but were not typical of Mongolism. The thumb to first finger distance was questionably enlarged. There was a deformity of the right ear. Diffuse, scattered rales were heard in the lungs. The heart appeared normal. When examined a short while later a systolic heart murmur was heard at the left sternal border, and for the first time the abdomen was noted to be distended by a mass in the epigastrium measuring 3 x 4 cm. and not freely movable. Intravenous pyelography revealed a 4 cm. round mass in the right retroperitoneal region, displacing the bowel anteriorly. There was faint visualization of dye bilaterally. The right pelvis appeared somewhat dilated, while the left side was normal. It could not definitely be concluded from the films

* Post-doctoral Fellow, Department of Pharmacology.

Received for publication 9 August 1961.

whether the mass was connected to the right kidney or not. Seventeen hours after birth the infant was in moderate respiratory distress, but she was not cyanotic as long as she remained in oxygen. The respiratory rate was 58. There was no jaundice, edema or rash, and hemoglobin was 17.5 gm.%. The systolic murmur at the left sternal border had increased in intensity to grade III. Thirty hours after birth the infant suffered labored respirations even in oxygen. Coarse and fine rales were heard over the right lung posteriorly. The reflexes had become more sluggish. The cardiac sounds became irregular with a gallop rhythm and a rate that decreased to 40. Suddenly, 36 hours after birth, the heart sounds became inaudible and the infant was pronounced dead.

NECROPSY FINDINGS

Gross Notes. The weight was 1900 gm., the crown-rump length 27 cm., and the crown-heel length 42 cm.

The body appeared well nourished and developed. Large portions of the right parietal and frontal bones consisted of thin membranous bone. The abdomen was flat, the external genitalia were those of a normal female infant.

On opening the thoracic cavity, the lungs were noted to be dark red-brown, and retracted from the chest cage. They had a rubbery consistency. The normal number of lobes was present. The parenchyma was homogeneous and showed no focal lesions. The bronchial and vascular trees revealed no abnormality. In situ, the heart was seen to be greatly enlarged, especially the right atrium and ventricle. The great vessels originated from and entered the heart normally except for the aorta, which appeared displaced somewhat to the right. The heart, together with the right lung, weighed 28 g. On opening the heart, a 4 x 5 mm. highly placed defect was seen in the interventricular septum. The aortic valve partially overrode this defect. Both the pulmonic and the aortic valves were bicuspid.

On opening the peritoneal cavity, a large cyst was seen to occupy almost half of the abdomen (Fig. 1). It was retroperitoneal, elongated and S-shaped, extending from the right kidney, along the midline down into the pelvic cavity (Figs. 2 and 3). The cyst wall appeared pale gray and was relatively translucent. Its thickness was estimated to be less than 1 mm. The contents were largely liquid, but on examining the specimen after formalin fixation, some gas was present. This was thought to have entered as a result of an accidental post mortem perforation of the cyst. The liquid, approximately 100 ml. in volume, appeared colorless or light yellow through the wall of the cyst, but was not examined further. The cyst was found to originate within the superior half of the right kidney (Fig. 4), which it left via a 5 mm. hollow stalk, separate from, and above the normally placed

major renal pelvis. The cyst then coursed under the mesenteries of the small and large intestines, under the right mesosalpinx and ovary into the broad ligament, where it appeared to end blindly. However, an apparently solid stalk of fibrous tissue was seen to continue to the superior portion of the vagina on the right side (Figs. 3 and 5). This stalk was 2 mm. wide and 2 cm. long.

Except for several small 1-3 mm. thin-walled cysts at the superior pole, the right kidney appeared completely normal, as did the left kidney. One ureter was seen to leave each kidney, and each was found to enter in proper position at the vesical trigone. The ovaries and Fallopian tubes were symmetrical and appeared to be normal, as did the uterus. In the vagina a cystic elongated ridge was seen in the innermost portion on the right side, close to the attachment of the large retroperitoneal cyst (Fig. 5).

No abnormality of the gastrointestinal tract was noted except for a Meckel's diverticulum (Fig. 1), which was 2 cm. long. The anus was normally patent.

The liver, spleen, pancreas and adrenal glands all appeared normal. No lesions were seen in the brain.

Microscopic Notes. Sections of the lungs showed alveoli to be filled with red and white blood cells, and vernix caseosa was detected focally. Except for the genitourinary system, the rest of the organs had a normal appearance. There was no evidence of infection.

Microscopic examination of the left kidney and the lower pole of the right kidney failed to reveal anything unusual for the age of the child. Thus, the glomeruli and tubules were noted to be highly cellular, containing cuboidal to columnar epithelium with large, prominent nuclei.

In the upper pole of the right kidney numerous cortical cysts were seen. These varied in size between one and six times the size of an average glomerulus and contained homogeneous, very faintly eosinophilic material. The lining consisted of a simple cuboidal epithelium, with darkly basophilic, closely packed nuclei. A layer of connective tissue of minimal thickness surrounded the cyst epithelium. Some of the cysts showed partial loss of epithelium.

The lumen of the thin stalk entering the upper pole of the right kidney was almost entirely denuded, but there were remnants of a cuboidal epithelium. The remaining cells had rather deeply eosinophilic cytoplasm, and large, moderately basophilic nuclei.

Serial sections were made in order to follow the stalk into the kidney substance. The stalk was seen first to form a pelvis-like structure (Fig. 6), into which it was possible to follow (except for short gaps), collecting ducts,

FIG. 1. Mesonephric duct cyst (C), viewed in situ. A Meckel's diverticulum (D) is seen in the upper left hand corner.

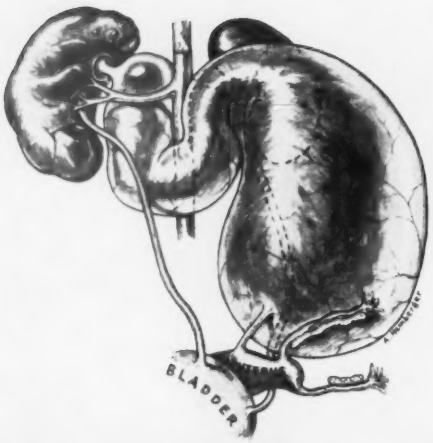


FIG. 2. Drawing of anterior view of mesonephric duct cyst. The intestines and their mesenteries have been removed to permit better visualization.

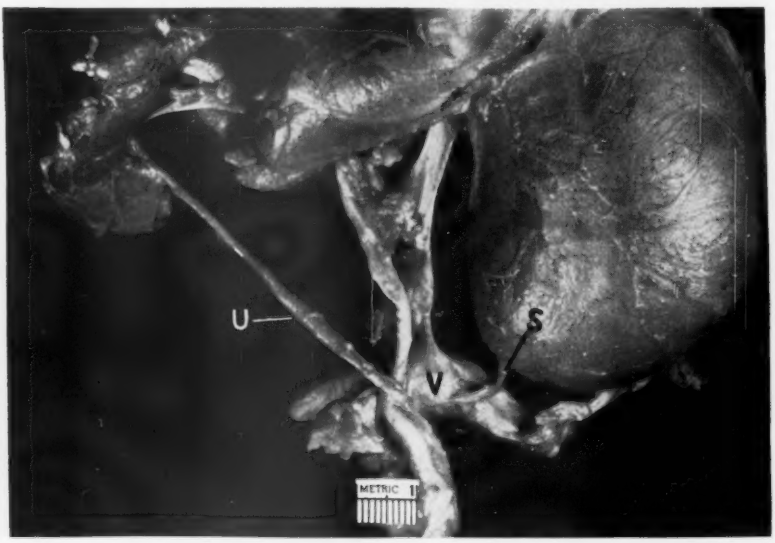


FIG. 3. Anterior view of mesonephric duct cyst, displaced toward the left, showing superior attachment to right kidney, and inferior attachment, by way of a solid stalk (S), to the right side of the vagina (V). In the lower right hand corner the right ovary and Fallopian tube are seen. Leading from the right kidney, the ureter (U) is seen prominently.

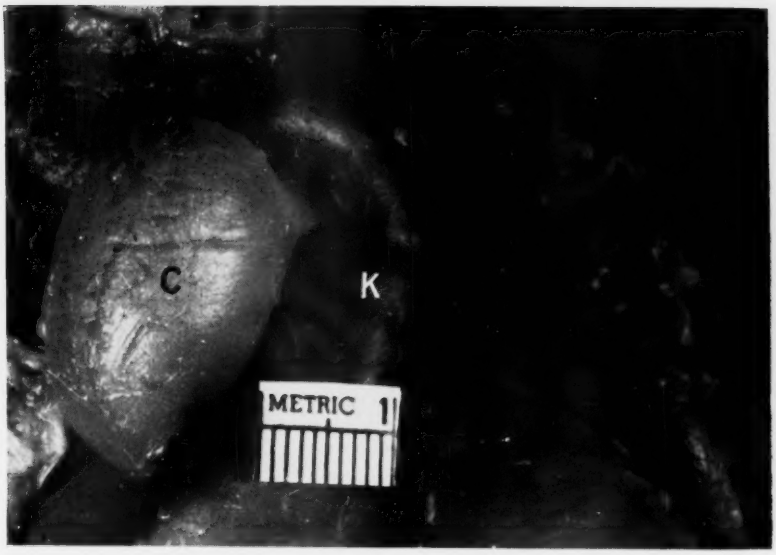


FIG. 4. Close-up of origin of mesonephric duct cyst (C) emerging as a pelvis-like structure from upper pole of right kidney (K), (posterior view).

FIG. 5. Close-up to show the stalk (S) connecting the mesonephric duct cyst (C) to the vagina (V). The lining membrane of the vagina is elevated by a series of cystic structures (arrows) distal to the attachment of the stalk.

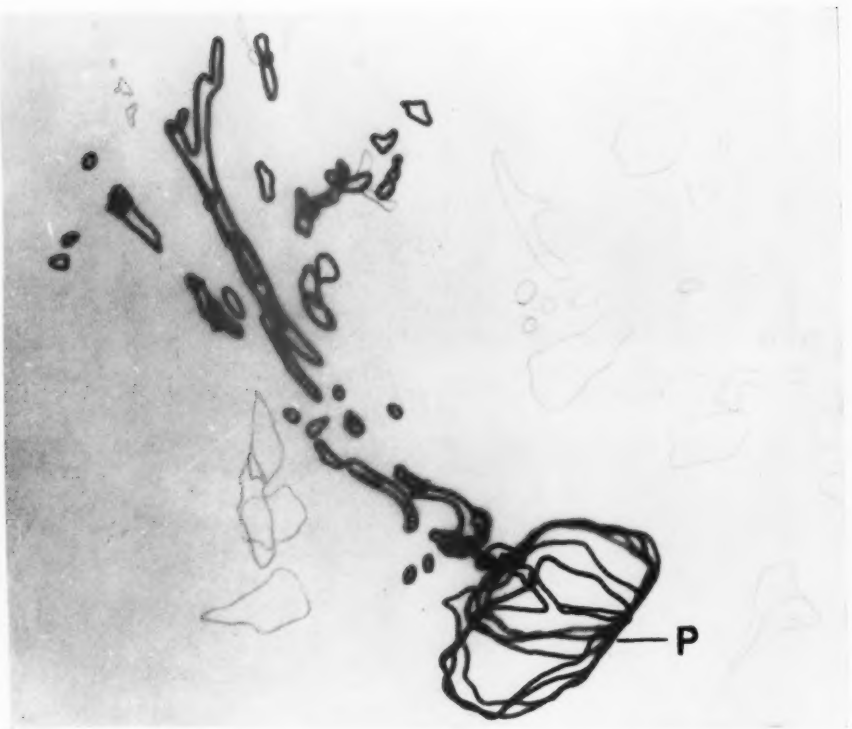


FIG. 6. A composite of several tracings of pelvis (P) and collecting ducts of mesonephric duct system, the composite having been made from selected serial sections.

originating near the capsular portion of the cortex. The pelvis-like structure was partially lined by simple, low cuboidal epithelium, similar to that of the stalk, and was also partially denuded of an epithelial lining. It was surrounded by areolar connective tissue which was denser in the immediate neighborhood of the pelvis. Within the areolar tissue there were numerous focal mononuclear infiltrates. In addition to the pelvis and collecting ducts leading to it, this portion of the kidney also contained large cystic structures, lined by a flat epithelium. These were interpreted as lymphatic channels.

The collecting ducts were lined by a tall columnar pseudostratified epithelium. They were straight, ran perpendicular to the surface of the kidney, and had several generations of branches. It was not determined whether these were ultimately connected with glomeruli.

Except for these collecting ducts, both the medulla and cortex of the right upper pole appeared normal for the infant's stage of development. The wall of the abdominal portion of the cyst was seen to consist of a thin areolar connective tissue lined by epithelium which had, in part, sloughed. What remained resembled the stalk epithelium. The serosa was a flat cuboidal single layer of cells, with the nuclei fairly far apart, and with faintly basophilic cytoplasm.

Within the superior portion of the vagina an elongated cyst-like space was seen, lined by a columnar, simple epithelium like that of the retroperitoneal cyst. Portions of this epithelium were also absent.

Both ovaries appeared normal microscopically with numerous well formed primordial follicles.

DISCUSSION

The evidence that this large cyst represents the mesonephric duct is as follows:

i) The cyst was retroperitoneal throughout its course.

ii) There were other urogenital anomalies present on the same side, namely cysts in the wall of the vagina and several cysts in the superior pole of the right kidney.

iii) On the right side, the ovary, the ureter, and the lower and middle portions of the kidney were all normal, suggesting that the cyst is distinct in origin.

iv) The cyst originated within the kidney parenchyma, which makes a nephrogenic origin most likely.

v) Because of its origin immediately adjacent to the metanephros within the abdomen, it is almost certainly not pronephric in origin. Pronephric

tubules are never found below the first thoracic segment,[8] and a migration of a rest down to the level of the definitive kidney is unlikely, although proposed by Paetzel.[9] Localization in the superior pole is common to mesonephric tissue and metanephric parenchyma drained by a supernumerary ureter. Unless a Gärtner's duct or other mesonephric duct remnant is found as well, it would seem that a supernumerary conduit from the superior pole of the kidney could equally well be interpreted as a mesonephric duct or a supernumerary ureter. (In the male, of course, such ambiguity would not exist, except in the absence of testis and spermatic cord.)

vi) In its course, which is essentially limited to the right half of the abdomen, the cyst coincided with several frequent locations for mesonephric duct remnants. Noteworthy in this respect were the close association with the right ovary, the attachment to the side of the superior vagina, and the terminal cysts in the wall of the vagina.

vii) There was no abnormality of the contralateral kidney, liver or pancreas. A polycystic kidney would therefore be unlikely.

SUMMARY

A large retroperitoneal cyst in a newborn female infant is described. Its origin, course and distal attachment together with associated findings are interpreted as evidence that it represents the mesonephric duct. A survey of the literature reveals such a cyst to be highly unusual.

ACKNOWLEDGMENTS

The constant interest and assistance of Dr. Averill A. Liebow in the preparation of this paper is gratefully acknowledged. Figure 2 was drawn by Mr. Armin Hemberger.

REFERENCES

1. Hamilton, W. J., Boyd, J. D., and Mossman, H. W.: *Human Embryology.* Baltimore, Williams and Wilkins Co., 1952.
2. Ashhurst, A. P. C. and McGuire, J. H.: A case of retroperitoneal cyst, probably originating in the Wolffian body. *J. Amer. med. Ass.*, 1920, *75*, 1494-1495.
3. Funfack, M.: Beitrag zur Kenntnis cystischer Hemmungsbildungen aus fruhembryonalen Nierenanlagen. *Z. Urol.*, 1956, *49*, 262-287.
4. Haslinger, K.: Eine multilokulare Nierenzyste. *Wien. klin. Wschr.*, 1926, *39*, 534-535.
5. Hennig, O.: Uberbleibsel von Urnierenanlagen. *Z. Urol.*, 1954, *47*, 493-508.
6. Klosterhalfen, H.: Zur Diskussion persistierender Urnierenanlagen. *Z. Urol.*, 1959, *52*, 31-35.
7. Maury, J. M.: Retroperitoneal cysts of Wolffian origin. *Surg. Gynec. & Obstet.*, 1918, *26*, 663-668.
8. Sander, E.: Ein Beitrag zur Kasuistik der persistierenden Urnierenanlage. *Z. Urol.*, 1956, *49*, 654-660.
9. Paetzel, W.: Persistierende Vorniere. *Z. Urol.*, 1949, *42*, 156-160.

THE PARATHYROIDS. Roy O. Greep and Roy V. Talmage, Eds. Springfield, Illinois, Charles C. Thomas, 1961. 473 pp. $12.25.

This volume contains the verbatim proceedings of a symposium on current research on the parthyroids held at Rice University early in 1960. Most of the papers which comprise the meat of the conference have already appeared, or are now appearing in print, in more conventional scientific journals. Nevertheless, it is a great help to the physiologist, biochemist, or clinician interested in this fascinating gland to have the cream of the most recent advances in parathyroid research available in a single volume. There is, happily, a minimum of loose talk in the final edited version about "preliminary results," the latter defined by W. F. Neuman as, "Preliminary means we have not done it twice." It is a tribute to the editors and organizers of the symposium that so much solid and new information is here, and so little extraneous conversation.

Among the high points of these reports are the elegant studies of Gaillard on the effect of parathyroid hormone on bone in tissue culture (osteoblasts vanish and osteoclasts appear and go to work). Aurbach and Rasmussen have further purified the hormone. It appears that, starting with a phenolic or acidic extract of the glands, a family of polypeptides can be obtained, ranging in size from 3,700 to 8,600 in molecular weight, all of which have *both* calcium-mobilizing *and* phosphaturic properties. In addition to improved chemical purification and analysis, further advances in parathyroid physiology clearly depend on more sensitive methods for bioassay. This problem is competently discussed by Paul Munson.

Although it has long been known that the parathyroids have a direct action on mobilization of calcium from bone and on the excretion of phosphorus by the kidney, it has only recently become apparent that they influence the handling of calcium by gut and kidney as well. Cramer, Sinker, and Copp present evidence that parathyroid hormone increases intestinal absorption of calcium. Kleeman shows in humans that parathormorne increases the renal tubular reabsorption of calcium, an effect already demonstrated in mice and rats by Talmage.

The relationship of parathyroid hormone to citric or lactic acid is discussed in four papers. This is an intriguing subject because of the capacity of these ions to chelate calcium (and hence, perhaps, increase its solubility at the surface of bone crystals) and because of their ubiquity in metabolizing tissues. W. F. Neuman has a stimulating chapter on possible fundamental mechanisms of action of the hormone.

In contrast to the new biochemical and physiological information about the parathyroids, there is nothing startling in the way of new clinical information in this book. All the "clearance" tests for hyperparathyroidism give the right answer when the diagnosis is already known—and fall down in just those cases where they are needed most. John Eager Howard describes a motheaten appearance of the acromioclavicular joint which he has found a helpful radiological sign. Absence of the lamina dura, often quoted as diagnositc, "has led us astray more than it has been of help."

This book is a valuable compendium for the research worker or teacher interested in parathyroid physiology. Though it will soon be outmoded, it is an important benchmark in an interesting story of continued progress.

FRANKLIN H. EPSTEIN

BONE: AN INTRODUCTION TO THE PHYSIOLOGY OF SKELETAL TISSUE. By Franklin C. McLean and Marshall R. Urist. Chicago, University of Chicago Press, 1961. xvii, 261 pp. $6.00.

The book is one of the series of *The Scientists Library: Biology and Medicine*. The purpose of the series is to provide authoritative information about the growth and status of various subjects in such a fashion that the individual books may be read with profit not only by the specialist but also by those whose interests lie in other fields.

The book is not intended as a full-scale monograph on the physiology of bone. Historical treatment is reduced to a minimum, documentation is limited, illustrations are few. On the other hand, the text provides an extended treatment of the current state of the literature. The authors are well qualified because both have made notable contributions to the knowledge of bone. In addition, they collaborated in a number of studies.

The content of the book is concerned with bone as a tissue rather than with its mechanical function in providing the skeletal support of the body or with the shape, adaptation, or development of individual bones. The intent of the authors is to bridge the gaps remaining between the various disciplines and to contribute to the unification of the subject. Nearly every approach to the study of bone is contained in the book including morphology, experimental embryology, histochemistry, enzyme chemistry, crystallography, endocrinology, electron microscopy, autoradiography, and microradiography. Complementing the many clinical treatises on bone, the book considers disorders of metabolism from the standpoint of pathological physiology rather than diagnosis and treatment.

Reflecting the progress in the study of bone, this second edition represents an extensive revision entailing a considerable amount of complete rewriting and expansion of most of the text. However, the book still remains a concise statement of the present status and important advances in its field.

E. S. CRELIN

HORMONES IN BLOOD. C. H. Gray and A. L. Bacharach, Eds. New York, Academic Press Inc., 1961. 655 pp. $20.

The purpose of the authors was to bring together, in a meaningful manner, the important data on approximately 15 groups of hormones found in human blood or plasma. The 22 contributors from seven different countries are authorities in their fields. For each group of hormones, a description of its chemical nature, biosynthesis and physiology, and evaluation of methods of measurement is given. The chapters on insulin, thyroid hormones, and the adrenal steroids are thorough, up to date, and cite important references

in their respective fields. The chapters on adrenocorticotrophin, oxytocin, and vasopressin are not very inspiring.

This book will be a useful reference book for research endocrinologists or those involved in the measurement of hormones in blood. The latter will find it useful for its critical evaluation of methods rather than a description of a particular method. It is a good book, well written, and, in general, the authors have accomplished their purpose.

PATRICK J. MULROW

CARCINOGENESIS. By I. Hieger. Chester Beatty Research Institute, London. London and New York, Academic Press Inc., 1961. xi, 138 pp. $5.

In this volume the author planned to put "into perspective the theories which have been advanced to interpret the process whereby normal cells and tissues are transformed to cancer." To this end he has quoted extensively in and sometimes possibly somewhat out of context from those who have been inclined to theorize on what cancer is and how it comes about. One cannot doubt that "quotations are authentic statements" in that they can be ascribed to an author, but authenticity and truth are not identical. Often of course the statements were concepts or interpretations at best and not presented as 'truths.'

The small volume is nicely and provocatively written. It follows a historical outline, in general, in that the tissue balance, irritation, embryologic rest, somatic mutation, and immunological theories or hypotheses of the causes of cancer are taken up in order. Only the last two are of greatest contemporary concern and are discussed in some detail. The general conclusion is that they merit no more than the status of theories. In the absence of scientific proof they are at best working hypotheses or extrapolations and at worst flimsy reasoning or tautologies. It should be obvious that the multiplicity of extant theories probably means that all are inadequate.

One chapter concerned the carcinogens is almost exclusive summarizing and description rather than analytical or critical except insofar as the author has been selective. This is followed by a brief chapter on intramolecular electronic configuration and carcinogenesis. The difficulty of evaluating carcinogenicity quantitatively in a biological sense has frustrated attempts to determine physical-chemical characterization which might correlate with biological activity. The few nice studies which attempt to localize the site of action of carcinogens at subcellular or mechanistic levels are inadequate for generalization at this time.

Carcinogenesis has been assumed by some to be a multi-stage process, (Chapter 5) for example, an initiating stage and a promoting stage. Substances or stimuli called initiators or promoters are associated with these stages. These are but terms associated with gross biological manifestations under special conditions and add little to understanding the processes acting at basic and cellular levels. That an 'initiator' is a stimulus or stimulator that initiates a response in a tissue organism or cell that when promoted by a 'promoter' leads to cancer, is itself an interpretation.

The observations of carcinogenesis *in vitro* (Earle, *et al.*) and of anaerobic glycolysis (Warburg) in cancer are discussed briefly. Professor Hieger is particularly impressed by carcinogenesis *in vitro* but was not convinced that metabolic change was, at best, more than a concommitant of cancer rather than the cause.

Each investigator tends to look at cancer through glasses tinted by the shades of his discipline or experience; "Huxley is compelled to see cancer through the eyes of an evolutionist." No theory "so far advanced has been able to cope with the difficulties and contradictions" which appear when all the data are "closely examined by logical, observations or experimental means." What has seemed logical and important to Dr. Hieger has been presented in this small volume. Dr. Hieger has been working on problems of carcinogenesis and carcinogens for over 30 years and has an experienced background that merits his consideration of cancer research "as a problem of pathology" in this volume. The reviewer must agree that the only reason for including the six electron micrographs is that "experimental cancer could hardly be considered complete without photographs of what the author is writing about."

W. U. GARDNER

MATHEMATICAL PRINCIPLES IN BIOLOGY AND THEIR APPLICATIONS. By Nicholas Reshevsky. Springfield, Ill., Charles C. Thomas, 1961. 128 pp. $6.

The author states that this book is addressed to "those biologists and physicians who have a general knowledge of mathematics but who are not sufficiently at home with it to read the more technical publications in this field." In order to attempt to rectify this situation, a number of very brief chapters are offered, ranging from "Diffusion Processes" through "Shapes and Numbers" to "Some Notions of Topology." The account of diffusion is short and to the point. If the reader understands the use of partial differentials, he will have no difficulty. Probably he will have no great interest, either. The typical medical reader is not likely to "feel at home" with partial differentials, and it seems doubtful that his unfamiliarity will be changed sufficiently after reading this portion of the book. Later chapters have titles such as "Excitation and Inhibition," followed by "Applications." Basically these chapters deal with exponential functions and simple expansions, but these fundamental quantities tend to be lost in a welter of "excitations," "reaction times," etc. This reviewer feels that the fundamental difficulty is one familiarity with the exponential function; once this is gained, the applications present no great problems.

The last part of the book is concerned with Sets and Topology. If the reader is not very facile with exponentials, partial differentials, etc., it seems most unlikely that he will have any general knowledge of Set Theory or Topology. The fundamental nature of these subjects cannot be disputed, but for the avereage medical reader these chapter will serve no purpose whatever, except perhaps to confirm in his mind the idea that mathematics belongs elsewhere. The space would have been better devoted to discussion

of, say, the meaning and significance of first order differential equations or the use of series expansions for the approximation of functions.

In general this small book will not be of great technical assistance to the non-mathematical reader. To those whose concept of mathematics begins to falter at the Henderson-Hasselbach equation, it may serve a purpose in showing that mathematics has nothing to offer in the life sciences.

S. J. FRICKER

SOME RECENT DEVELOPMENTS IN THE CHEMISTRY OF PHOSPHATE ESTERS OF BIOLOGICAL INTEREST. By H. Gobind Khorana. New York, John Wiley & Sons, Inc., 1961. x, 141 pp. $5.25.

This book presents to a wide audience a series of lectures given by H. G. Khorana at the Rockefeller Institute in 1959. Few others could have matched the qualifications of the author to speak about recent developments in the chemistry of phosphate esters of biological interest. They have occurred to a major extent in Khorana's laboratory.

The book is surprisingly comprehensive considering its small size and the rapid advances made currently in this particular field. It is nevertheless clearly oriented toward the author's special fields of interest. It starts with a classification of phosphate esters and a review of the methods for phosphate ester synthesis. A discussion of cyclic phosphate chemistry leads then to an account of the chemistry of the phosphate groups of nucleotides and polyucleotides. The book closes with a review of the synthetic applications of carbodiimides. This final chapter touches on their use in the synthetics of compounds not containing phosphorus.

Primarily, the book is a reference monograph. It is replete with practical suggestions and comments on reaction conditions as they affect yield, selectivity, and stability. It is well documented with a bibliography at the end of each chapter. The coverage of literature is practically up to the minute. A general discussion of the mechanisms of the various reactions and of the factors affecting them is included. This enhances the volume's usefulness to those who are in need of help in particular cases not covered in the book explicitly. It is unfortunate that the book has no index, although the table of contents at the head of each chapter enables the user to find specific details relatively quickly in this small volume.

The preface offers the book to chemists and biochemists in general, not alone to those working in the special field of phosphate biochemistry. The generalizations and the discussions of reaction mechanisms indeed should be of interest to all, but the wealth of detail may fail to hold the attention of many. In any case, the very liberal use of structural formulae should ease considerably the rapport between the author and the general reader.

With due allowance for the title, implying a *selective* treatment of developments, it is regrettable perhaps that a book on phosphate ester chemistry should not include any discussion of the hydrolysis of phosphate esters except insofar as cyclic phosphates play a role in such hydrolyses.

This remark notwithstanding, Khorana's book is a very welcome and highly useful volume on the phosphate chemist's shelf and in the chemical and biochemical library, comprehensive within the scope set by the author, and vivid and authoritative as a result of his so eminent involvement in the recent advances of the field under his review.

G. TABORSKY

PROBLEMS OF PULMONARY CIRCULATION. A. V. S. de Reuck and Maeve O'Connor, Eds. Ciba Foundation Study Group No. 8. Boston, Little Brown & Company, 1961. 96 pp. $2.50.

This small book consists of a series of papers dealing with various aspects of hemodynamics in the macro-circulation of the lungs. The papers have little to say about the micro-circulation or the structure of the vessels themselves. Each paper is followed by a spirited discussion which proves to be a salubrious force against bias. The study group includes some of the most able and experienced investigators in the field. Its members represent an interesting balance between the clinical physiologist and the classical experimental physiologist. Dr. André Cournand, in his opening remarks as chairman, emphasizes the great value of the clinical physiologist's observations, then adds, "but it is important that these observations be then analyzed by the experimental method." The authors present this balance of observation and experimentation, and integrate the data into principles of pulmonary circulation so successfully that the novice reader will gain an understanding in depth as well as breadth with a small expenditure of reading time.

Various methods of assaying the hemodynamic phenomena of the pulmonary circulation are presented and a great deal of cogent discussion centers about the neuro, chemo, and humoral control of the pulmonary circulation. Perhaps the most exciting presentation is that of the group from Postgraduate Medical School who have developed the radioactive oxygen-15 technique for estimating regional ventilation and perfusion. Although no more important than the other papers for the general field of pulmonary physiology, this is an interesting new technique which represents the conquest of a hitherto unsolved problem.

FRANK D. GRAY, JR.

EVALUATION OF DRUG THERAPY. Francis M. Forster, Ed. Madison, University of Wisconsin Press, 1961. 167 pp. $4.

This is a report of a symposium on the methodology of evalution of drug therapy in neurological disease. It was held at the University of Wisconsin and was supported by a grant from the U.S.P.H.S. The role of the pharmacologist, the pharmaceutical industry and the Food and Drug Administration is presented in the first section, that of the biostatistician in planning controlled clinical trials in the second, and reports of he panels on various disease entities in the last.

The participants in the first section discuss the value and the limitations of animal screening and the ultimate importance of controlled clinical

testing in determining the usefulness and safety of a new drug. This section provides orientation for the uninitiated. The excellent section on controlled clinical trials indicates the hard work and attention to detail which must go into their planning and conduct if subsequent mishaps are not to lead to great waste of effort. It points out the importance of defining the disease to be studied and of establishing criteria for measuring response to therapy which are reproducible and generally acceptable. It indicates that the aim of controlled testing is comparison, not necessarily with a placebo, but often better with an accepted modality. It discusses the randomization of treatment groups, blind procedures, the number of patients necessary to give significant results and the conditions in which cooperative ventures involving several institutions may be necessary. In the last section, panels of clinicians, each with its biostatistician, attempt to plan clinical trials in relation to particular diseases.

It seems to the reviewer that this symposium was of great value to the participants and to others who contemplate trial of therapy in neurological disease. The need for controlled testing is great, the reward small. If the symposium stimulates only a few competent workers to do or promote proper clinical trials, it will have been well worth while.

PAUL LAVIETES

GENETICS: GENETIC INFORMATION AND THE CONTROL OF PROTEIN STRUCTURE AND FUNCTION. Transactions of the first conference, October 1959. H. Eldon Sutton, Ed., New York, Josiah Macy, Jr. Foundation Publications, 1960. 229 pp. Illus. $6.

This is a direct transcript of the first in a series of annual invitational conferences on Genetics sponsored by the Macy Foundation. It had as its underlying theme the following "dogma": "A DNA code specifies an RNA code which determines the amino acid sequence of a particular protein" (p. 177). According to Cyrus Levinthal, ". . . . the reason we call this [a] 'dogma' is that it depends on personal bias, not logic" (p. 107).

Three discussion sessions were held. The first which was opened by J. D. Watson, although entitled "Current Concepts of the Fine Structure of the Chromosome and the Nature of the Coding Mechanisms," was in reality an evaluation of current knowledge regarding the physical structure of DNA. In the concluding minutes, some attempt was made, without notable success, to relate this information to chromosome behavior. There is a brief but valuable summary by Joshua Lederberg, pointing out the pitfalls involved in current laboratory manipulations of DNA and suggesting areas in which more research is urgently needed to clairfy the relationships between cytogenetic phenomena and the physical and chemical characteristics of DNA.

The second session, "The Genetic Control of Protein Structure," was led by V. M. Ingram. Speculations regarding the coding mechanism and protein structure were discussed in considerable detail by several participants in terms of current research on intermediate steps in protein synthesis, the structure and characteristics of normal and inherited variants

of human hemoglobins and haptoglobins, and various microbial enzyme systems. There is also a succinct discussion by Levinthal of current alphabetical models of the coding system.

Mechanisms which regulate the activity of proteins produced through the series of events postulated by the dogma were considered by S. Spiegelman in the final session "Factors Modulating the Biochemical Expression of Genetic Systems." Of the four control mechanisms suggested, three, that is, feedback inhibition, feedback repression, and enzymatic induction, have been experimentally demonstrated; the fourth, control of the number of protein synthesizing machines, is hypothetical at present.

The considerable contributions that have been made by the work in microbial genetics on one hand, and in human biochemical genetics on the other, contributions to our knowledge of the relationship between gene structure and protein structure, were emphasized throughout the Conference. It is also apparent from this Conference that great gaps still exist between that which is known of the physical and chemical nature of the hereditary material and the observed facts of chromosome behavior.

Although this small book betrays, in varying measure, a lack of unity, emphasis, and coherence, such flaws which are inherent to the direct transcription of informal discussions, it also has the charm of unexpected flashes of wit, unconscious humor, and the occasional disclosure of the human side of some eminent scientists. Geneticists and cytologists will find much that is of great interest in this book, and the two final chapters should be thoughtfully perused by any biologist interested in cellular mechanisms underlying basic development phenomena.

S. J. COUNCE

BOOKS RECEIVED FOR REVIEW

Books received are acknowledged in this department, and such acknowledgment must be regarded as a sufficient return for the courtesy of the sender. Selections will be made for review in the interests of our readers and as space permits.

The Story of X-Rays from Rontgen to Isotopes. By Alan Ralph Bleich. New York City, Dover Publications Inc., 1961. 186 pp. $1.65.

Report of the Commission on Enzymes of the International Union of Biochemistry. Oxford, Pergamon Press Ltd., 1961. 160 pp. $7.50.

Introduction to Biochemistry. By E. O'F. Walsh. New York, The Macmillan Company, 1961. 454 pp. $7.50.

The Fire of Life. By Max Kleiber. New York, John Wiley & Sons, Inc., 1961. 454 pp. $11.50.

Immunity, 2nd Edition. By Sidney Raffel. New York City, Appleton-Century-Croft, Inc., 1961. 672 pp. $10.00.

Metabolic Pathways in Microorganisms. By Vernon H. Cheldelin. New York City, John Wiley & Sons, Inc., 1961. 91 pp. $3.50.

Man, Time, & Foccils, 2nd Edition. By Ruth Moore. New York City, Alfred A. Knopf, Inc., 1961. 436 pp. $6.95.

The Chemistry of the Injured Cell. By Sir Roy Cameron, and W. G. Spector. Springfield, Ill., Charles C Thomas, 1961. 147 pp. $6.00.

Microtechniques of Clinical Chemistry, 2nd Edition. By Samuel Natelson. Springfield, Ill., Charles C Thomas, 1961. 578 pp. $4.75.

The Science of Genetics. By Charlotte Auerbach. New York City, Harper & Brothers, 1961. 273 pp. $5.95.

The Compleat Pediatrician. By Wilburt C. Davison & Jeana Davison Levinthal. Durham, N.C., Duke University Press, 1961. 257 pp. $4.50.

Genes, Enzymes, and Inherited Diseases. By Eldon Sutton. New York City. Holt, Rinehart & Winston, Inc., 1961. 120 pp. $3.50.

British Medical Bulletin, Human Genetics, Volume Seven, No. Three, September 1961. London, Medical Department of The British Council. 177-264 pp. $3.25.

Supersensitivity Following Lesions of the Nervous System. By George W. Stavraky. Toronto, University of Toronto Press, 1961. 210 pp. $10.00.

Dictionary of Aphrodisiacs. By Harry E. Wedeck. New York City, Phillosophical Library, Inc., 1961. 256 pp. $10.00.

Sensory Communication. Edited by Walter A. Rosenblith. New York & London, John Wiley & Sons, Inc., and Cambridge, The M. I. T. Press, 1961. 844 pp. $16.00.

Darwinism and the Study of Society, A Centenary Symposipm. Edited by Michael Banton. Chicago, Quadrangle Books, Inc., 1961. 191 pp. $5.00.

Bioelectricity. By E. E. Suckling. New York, Toronto, London, McGraw-Hill Book Company, Inc., 1961. 233 pp. $8.75.

Oxytocin. Edited by R. Caldeyro-Barcis & H. Heller. Oxford, Pergamon Press, Ltd., 1961. 443 pp. $15.00.

Essentials of Histology. By Gerrit Bevelander. St. Louis, The C. V. Mosby Company, 1961. 288 pp. $5.75.

Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest—4450. By H. Gobind Khorana. New York 16, John Wiley & Sons, Inc., 1961. 141 pp. $5.25.

The Machinery of the Body. By A. J. Carlson, Victor Johnson and H. Mead Cavert. Chicago, The University of Chicago Press, 1961. 752 pp. $6.50.

Carter's Principles of Microbiology. By Alice Lorraine Smith. St. Louis, The C. V. Mosby Company, 1961. 603 pp. $6.00.

Virus Meningo-Encephalitis, Ciba Foundation Study Group No. 7. Edited by G. E. W. Wolstenholme & Margaret P. Cameron. Boston, Little, Brown & Company, 1960. 120 pp.

Problems of Pulmonary Circulation, Ciba Foundation Study Group No. 8. Edited by A. V. S. de Reuck & Maeve O'Connor. Boston, Little Brown & Company, 1961. 96 pp. $2.50.

Health and Fitness in the Modern World—A collection of papers presented at the Institute of Normal Anatomy and the Ministry of Foreign Affairs, Rome, Italy, Leonard A. Larson, Conference Chairman. Chicago, The Athletic Institute, 1961. 392 pp. $4.50.

Quinones in Electron Transport, Ciba Foundation Symposium. Edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. Boston, Little Brown and Company, 1960—May. 453 pp. $11.00.

The Nature of Sleep, Ciba Foundation Symposium. Edited by G. E. W. Wolstenholme and Maeve O'Connor. Boston, Little Brown & Company, 1960 (June 27-29). 416 pp. $10.00.

Mathematical Principles in Biology and Their Applications. By Nicholas Rashevsky. Springfield, Ill., Charles C Thomas, 1961, 128 pp. $6.00.

The Parathyroids. Edited by Roy O. Greep and Roy V. Talmage. Sprinfield, Ill., Charles C Thomas, 1961. 473 pp. $12.25.

Human Pituitary Gonadotropins. Edited by A. Albert. Springfield, Ill., Charles C Thomas, 1961. 545 pp. $16.75.

Basic Biochemistry. By M. W. Neil. Philadelphia, J. B. Lippincott Company, 1961. 360 pp. $6.75.

Heredity—An Introduction to Genetics. By A. M. Winchester. New York, Barnes and Noble, Inc., 1961. 269 pp. $1.75.

Human Genetics. By C. C. Li. New York, McGraw-Hill Book Co., Inc., 1961. 218 pp. $8.50.

The Rale of Speech in the Regulation of Normal and Abnormal Behavior. By A. R. Luria. New York & London, Pergamon Press, Inc., 1961. 100 pp. $8.50.

Subluxation and Distortion of Joints Without Fracture. By Rowland Hill Harris. New York, University Publishers Inc., 1961. 156 pp. $6.00.

Preventive Medicine in World War II, Volume V, Communicable Diseases. Edited by Ebbe Curtis Hoff. Washington 25, D.C., Office of the Surgeon General, Department of the Army, 1960. 530 pp. $5.75.

Hormones in Blood. By C. H. Gray & A. L. Bacharach, London, Academic Press, Inc., (London), Ltd., 1961. 655 pp. $20.00.

Unresting Cells. By R. W. Gerard. New York, Harper & Brothers, 1961. 434 pp. $2.25.

The Social Insects. By O. W. Richards. New York, Harper & Brothers, 1961. 219 pp. $1.50.

Animal Ecology. By W. H. Dowdeswell. New York, Harper & Brothers, 1961. 209 pp. $1.50.

Darwin's Finches: An Essay On the General Biological Theory of Evolution. By David Lack. New York, Harper & Brothers, 1961. 204 pp. $1.40.

The Evolution of Human Nature. By C. Judson Herrick. New York, Harper & Brothers, 1961. 500 pp. $2.45.

Cell and Psyche, The Biology of Purpose. By Edmund W. Sinnot. New York, Harper & Brothers, 1961. 119 pp. $.95.

THE YALE JOURNAL OF BIOLOGY AND MEDICINE

333 Cedar Street. New Haven 11, Conn.

Scope. THE YALE JOURNAL OF BIOLOGY AND MEDICINE publishes original contributions in all fields of medicine, the fields of biology that are related to medicine, and in the history and teaching of these subjects.

Mailing address. Manuscripts should be addressed to THE YALE JOURNAL OF BIOLOGY AND MEDICINE, 333 Cedar Street, New Haven 11, Connecticut, U.S.A. All manuscripts should be mailed flat.

Preparation of copy. Papers should be typed in double or triple space, with references typed *in double space* on a separate sheet. For the convenience of authors, as well as of the editors, certain conventions in style of citation have been adopted as followe: References will be listed in the order of citation in the text of the manuscript and numbered consecutively. In the text they will be cited by superior number without parentheses. The order of citation of journal articles will be: author's name (surname followed by initials or forename if there is no middle initial), title of article, abbreviated title of journal (underlined), year, volume number (underlined), and complete pagination. Titles of journals will be abbreviated in accordance with the list of *World Medical Periodicals* (Published by World Medical Association, 10 Columbus Circle, New York 19, New York. 2d ed., 1957). In case this publication is not available to an author, full titles of journals should be submitted without abbreviation. References to books will include the author's name, the title of the book, and the place of publication, publisher, and date, followed by the page or pages containing the information to which reference is made. Authors of historical papers may use the conventional footnote style.

Lucké, Baldwin and Rea, M. H.: Studies on aneurysm. *J. Amer. med. Ass.*, 1921, *77*, 935-940.

Barcroft, Joseph: *The respiratory function of the blood.* Cambridge, Cambridge University Press, pp. 58-61.

Illustrations. All illustrations and tabular matter must be in finished form for the printer. Illustrations must not be larger than 8½ by 11 inches; if the original is larger, it should be photographically reduced on a glossy print. Line cuts will usually be reduced for printing to 4½ inches or less in width. The artist should be informed of the anticipated reduction before the drawing is made. All illustrative material should have titles, and the legends should be on the material itself as well as typed on a separate sheet.

Each paper is accepted with the understanding that it is to be published exclusively in THE YALE JOURNAL OF BIOLOGY AND MEDICINE unless some other specific arrangement has been made in advance.

Costs chargeable to authors. The number of free illustrations will vary with the length of the paper, but usually only two pages of halftones will be allowed free. Costs of additional halftones will be charged to the author. Special typography, including tables and formulas, in excess of 10 per cent of composition costs are chargeable to the author. Corrections in galley proof in excess of 5 per cent of original composition costs will also be charged to the author. Page proof will not normally be sent to the author, but in the event that an author makes any changes from copy after galley proof has been submitted, the entire cost will be charged to him.

for the diarrheal attack

effective—eradicates enteric bacterial pathogens

selective—does not eradicate the normal intestinal flora[1]

FUROXONE® LIQUID

brand of furazolidone

New, convenient prescription size: bottle of 2 oz. Also: bottle of 16 oz.

■ Exceptionally broad bactericidal range includes species and strains now resistant to other antimicrobials ■ Virtually nontoxic ■ Does not encourage monilial or staphylococcal overgrowth ■ Has not induced significant bacterial resistance ■ Dosage may be found in your PDR.

FUROXONE LIQUID is a pleasant orange-mint flavored suspension containing FUROXONE 50 mg. per 15 cc., with kaolin and pectin.

1. Mintz, A. A.: Antibiot. Med. 7:481, 1960.

EATON LABORATORIES, Division of The Norwich Pharmacal Company, NORWICH, N. Y.

SUS-PHRINE

THE MOST USEFUL FORM OF EPINEPHRINE

THE DRUG OF CHOICE IN BRONCHIAL ASTHMA

sus-phrine is an aqueous suspension of epinephrine 1-200. 20% of the epinephrine content is in aqueous *solution;* 80% is in the form of an undissolved fine particle *suspension.*

advantages Injected subcutaneously, the dissolved 20% exerts practically immediate effect; the suspended portion is a repository dose, absorbed slowly, exerting its effect from 6 to 8 hours. Thus, a single injection of Sus-Phrine replaces a 1:1000 aqueous injection plus a painful intramuscular oil suspension.

clinical experience *SUS-PHRINE IS BEING USED WHEREVER EPINEPHRINE IS INDICATED:* asthma, angioneurotic edema, allergic reactions, urticaria, anaphylactic shock, drug reactions. Wherever Sus-Phrine has been used clinically, it has become the epinephrine preparation of choice. Over thirty published papers delineate the superiority of Sus-Phrine suspension, especially in pediatric practice.

supplied In 0.5 cc. ampuls, packages of 12. Also in 5 cc. multidose vials. Keep a supply of ampuls in your bag and office, and vials in the hospital emergency drug room.

REFERENCES: *1. Harris, Coleman M. (Textbook) 1958. 2. Sherman, Wm. B., and Kessler, Walter R.: Allergy in Pediatric Practice, Mosby (St. Louis) 1958. 3. Kary, Ross C., Picbek, Robert A., and Sternlieb, Richard O.: Comparative Evaluation of Epinephrine and Aminophyllin as Bronchodilator Agents. The Amer. Review of Tuber. and Pul. Diseases. Vol. 77, May 1958, 5:729. 4. Unger, L., Wolf, A.A., Johnson, J.H., and Unger, D.L.: Management of Bronchial Asthma in Children, J. Ped. 52:538 (May) 1958. 5. Modell, Walter: Drugs of Choice 1958-1959, p. 179, 188, 528, 607. Mosby (St. Louis). 6. Brown, E.A.: The Treatment of Ragweed Polinosis with a Single Annual Emulsified Extract Injection II, Ann. of Allergy, 16:281 (May-June) 1958. 7. Brown, E.A.: The Treatment of Tree Pollen Sensitivity with a Single Annual Injection of Emulsified Extract III, Ann. of Allergy, 16:553 (July-August) 1958. 8. Brown, E.A.: abstract of References 24 and 25. Mod. Med., 26:22 p. 185 (Nov. 15) 1958. 9. McLean, J.A.: Some Considerations in the Management of Bronchial Asthma. G.P., 18:132 (Dec.) 1958. 10. Siegel, S.C.: The Treatment of Bronchial Asthma in Childhood: Lederle Bulletin Symposium Report I, No. 3 (p. 43) 1958. 11. Current Therapy 1959. 12. Buffum, William P.: Management of Asthma in Infancy. Asthma in Infancy. Pediat. Clin. North America, 6:683-691, 1959. Excerpted in Mod. Med., Oct. 15, 1959.*

Brewer
Est. 1852

BREWER & COMPANY, INC.

WORCESTER 8 MASSACHUSETTS